CLINICAL IMMUNITY & SERO DIAGNOSIS

WOLFF-EISNER—MATSON

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AND

SERO-DIAGNOSIS

BY

A. WOLFF-EISNER, M.D.

BERLIN

TRANSLATED BY

RAY W. MATSON, M.D.

PORTLAND, OREGON, U.S.A.

PROFESSOR OF HISTOLOGY, NORTH PACIFIC COLLEGE OF DENTISTRY AND PHARMACY

PATHOLOGIST TO MULTNOMAH COUNTY HOSPITAL

PHYSICIAN TO PORTLAND FREE TUBERCULOSIS DISPENSARY

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AUTHOR'S PREFACE TO ENGLISH EDITION

Dr. Matson, one of my pupils, has translated this little volume into English, and has done it so thoroughly that the translation apparently corresponds to the original in every point.

I am much gratified with this English translation, especially so, since it makes the work accessible, not only to the German-speaking profession, but to almost the whole civilized world; and for none of my works do I wish the possibility of such extension more than for this modest little 'Clinical Immunity.'

The reason for my feeling in this matter is that the work represents the first attempt to establish a connection, previously lacking, between biology (particularly immunity) on the one hand, and the clinic (particularly general practice) on the other. The venture was unprecedented, for, apart from a brief brochure by Much, nothing had yet appeared on the subject. The undertaking was a happy one; the work met a very friendly reception in Germany, and many practitioners and clinicians have expressed their gratitude for the ideas and inspiration gained therefrom. Ehrlich himself, from the midst of the excitement at that time surrounding Salvarsan, informed me that the work seemed to him very fortunate and timely.

Meanwhile, two other works of a similar character have appeared, written by Citron and Much. I am glad to say that I find myself in entire agreement with the principles advocated, particularly by Much. The very reason I under-

took to write a work on immunity was that I believed that, with all the differences of opinion on various minor points, an agreement might be reached on the fundamental questions, so that it would be possible to lay before clinicians in a brief and concise form a summary of the work accomplished, asking that they on their part work with us. Such co-operation would be of far more practical value than the work apart from that of clinicians and practitioners. Very few who are doing research work in immunity are clinically trained, and, consequently, most of their work is done with animals; moreover, they are continually meeting with difficulties such that further clinical material is required before it is possible to proceed with the work.

I see, therefore, no disadvantage in the appearance of two similar works, but rather aid in attaining the end desired—viz., that immunity and its therapy may be permitted to enter into the clinic and practice, and that investigators in immunity may be given not only the theoretical laboratory work, but all the practical work and clinical material that they are willing and able to handle. Unfortunately this is done at present in only three places in Germany. If the interest of practitioners can be aroused in these important biological questions, there is room for a whole series of such works, albeit they will naturally show certain differences of opinion and preferences.

There was a distinct chemical and physical epoch, in which clinicians with a chemical or physiological training were called to the more prominent places. A similar era is now at hand, in which the leading chairs will be occupied by clinically trained investigators in immunity and biology, to the betterment of science and the good of humanity. This much-needed condition has been delayed because investigators—such as Koch and Ehrlich—were purely theorists, and in consequence the experimental-therapeutic institutes recently created in Germany are, without exception, connected with theoretical pharmacological institutes, whereas a part at least should be associated with University clinics and large hospitals.

This small work does not deal in detail with the various

questions it treats. Its purpose is chiefly to introduce the various subjects to the practitioner and to arouse his interest in them. I have thought to define its limits by the title, '*Clinical* Immunity.'

A certain subjectivity, said to attach to the work, has been criticized. It was my desire to avoid this wherever possible, and only give expression to such ideas when convinced by my own best judgment that subsequent developments would tend in the same direction. The publications of Much and Friedberger, and their acceptance in the scientific world, have proved to me that this view was correct, and that even now, more than a year after the publication of the German edition, it can no longer be said that the work is at all subjective.

In order to keep in view the object—*clinical* immunity—I have dealt but briefly with antitoxic immunity. Serumtherapy, so far as its special part is concerned, I have completely omitted, because I believe that no one individual can possess sufficient knowledge of all the preparations in question. This deficiency is very well supplied by a compilation edited by myself—'Handbuch der Serumtherapie,' Munich, 1910.

Although Dörr criticized the fact that 75 pages of this small work were devoted to the discussion of hypersensitiveness, I feel that it is rather an advantage to have recognized so early the pre-eminent clinical importance of the phenomena of this condition, and correctly, as many works on internal medicine published in the past year attest. We are at the beginning of an era of clinical research based on the laws of hypersensitiveness. If I have taken advantage of this state of affairs to prove, by quotation from publications which appeared between 1903 and 1911, that the present view of hypersensitiveness, its mechanism and clinical significance, was created by myself, only an ill-wisher could find fault with the fact.

I know very well that such citations should have no space in a small work, but he who understands and appreciates the frequent tendency of those who exploit the results of research to claim for themselves the credit for the fundamentals

on which they build, will admit that I had the right to include such evidence as was necessary to prove that I have claimed no credit which was not justly due me. (That my presentation is consistently objective anyone may easily prove for himself by reference to the works of Much, Sahli, Römer, and Kraus.)

So may this little work in its translated form go its way through the English-speaking world, and assist in realizing the end I so much desire.

DR. WOLFF-EISNER.

BERLIN,
August, 1911.

AUTHOR'S PREFACE TO GERMAN EDITION

This short work owes its origin to lectures given in the post-graduate courses of the Berlin *Dozentverein*. I felt that I had succeeded in imparting a knowledge of the phenomena of immunity to physicians who had been in practice but a relatively short time, arousing their interest in the subject, and convincing them of its practical importance. A number of physicians, among them clinicians, to whom the need of a simple orientation of this important and difficult subject seemed urgent, induced me to place these lectures within reach of a larger circle.

Whether the form I have chosen is as well adapted to a broader circle will be shown by the reception accorded this book. The need of a simplified presentation of this interesting and difficult subject is in itself a great one. The choice of works of this character is limited. Compends of many volumes are not to be considered. Römer's splendid presentation of the lateral-chain theory is more of a monographic character. Dieudonné's valuable work on immunity, vaccination, and sero-therapy deals more particularly with vaccination and sero-therapy, and Müller's lectures on immunity are lengthy and detailed, being better adapted for those who wish to investigate the subject more thoroughly.

The sentiment expressed by Deycke and Much, in their introduction to the newly-founded *Hamburgische medizinische-kritische Blätter*, has been used unconsciously as the aim of this small work: 'The whole science of immunity to be so simplified in technique and terms as to enable the practitioner to apply modern diagnostics and therapeutics in his work.'

Deycke and Much continue as follows: 'An understanding of modern works, particularly those on biological lines, is complicated by an over-popular tendency to introduce new

terms into literature. As an example of this we need only point to the subject of immunity, where language is becoming almost hieroglyphic, and where even an expert can scarcely grasp the terms, much less the ordinary practitioner. There is an indulgence in mental gymnastics, to use Kant's expression, which is not especially pleasing. And yet the great, controlling ideas—the little that we actually know, and with which leading men have achieved results in therapy —are of a relatively simple nature and easily understood. Such men as Jenner and Pasteur, Behring and Koch, have never enriched our scientific language by the formation of new words ; but they have given us scientific facts, and have shown us how to utilize the facts in order to protect and help mankind.'

In this work we have attempted, not to lay stress upon the well-known vaccination and serum-therapy, but simply to point out in a general way the clinical and diagnostic significance of modern immunity research.

A brief, concise form, leaving aside all details, even though they may invite discussion, conveys a certain subjectivity. This form has been chosen in order to enable the busy practitioner to become convinced of the practical significance of the phenomena of immunity. It is based upon the fact that the views advanced by me concerning toxins and endotoxins, hypersensitiveness, aggressins, and tuberculosis immunity are being widely circulated, sometimes with and sometimes without reference to my publications. An opportunity is thus afforded of showing, in the form of a general survey, that these views make possible a simplifying of many difficult problems, both didactically and technically.

Only in the highly important question of establishing laws of hypersensitiveness have I entered into sources, quotation from which permits the reader to draw his conclusions objectively. For that reason, also, subjects no longer claiming attention are dealt with the more concisely.

DR. WOLFF-EISNER.

POTSDAMMERSTRASSE 65, BERLIN, W.

TRANSLATOR'S PREFACE

During the time which I spent with the author of this book I was struck with his clear and precise manner of presenting the interesting but difficult subject of immunity. It was, therefore, with pleasure that I undertook the task of translating his work on the subject, and placing it within reach of my English-speaking colleagues.

The need of the general practitioner for a work offering an explanation of clinical phenomena from the standpoint of this branch of biology has long been felt, and if this volume serves its purpose, and enjoys only a fair measure of the popularity which has already been accorded the original in German, I shall feel amply repaid for my efforts.

I wish to take this opportunity to express my gratitude to Dr. Wolff-Eisner for his kind personal interest and many favours shown me in my work with him. I am indebted to him for the addition of a supplemental chapter to the one on Chemo-therapy, dealing especially with Professor Ehrlich's new preparation, '606.'

Much credit is due the publishers for many courtesies and valuable suggestions. I am under especial obligation to my secretary, Miss Mary L. Jeffery, for assistance in the work.

RAY W. MATSON.

Portland, Oregon,
July, 1911.

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Immunity and Sero-Diagnosis

CHAPTER I

Introduction—Infection and virulence.

Introduction.

The practitioner of to-day is impressed more and more with the need of familiarizing himself with the principles of immunity. It has become a question of moment in general practice, even for physicians who are unable to do active work in that line. For it is no longer possible to obtain a thorough knowledge of modern methods of diagnosis and treatment without knowing the fundamental principles. The principles of immunity have, to be sure, exercised their influence on therapeutics, and the great field of research has been enriched and prepared for therapy. Yet these have thus far been only tentative. Apart from the serum-therapy of diphtheria, the practitioner did not always need to take these investigations into account. Starting with false premises, and guided by one-sided theoretical assumptions, therapeutic measures have often been erroneous, the more so since in the greater number of reports a careful criticism of the clinical observations has been wanting. On the other hand, the significance of modern principles of immunity in diagnosis is irrefutable. We are fully justified in regarding internal medicine to-day as the domain of a splendid diagnostic art. In that most important branch of internal medicine, tuberculosis, we may certainly hope that an early diagnosis, made possible by the new methods, will

also accomplish direct therapeutic results, since improved diagnosis always leads to therapeutic successes. It will be evident throughout this work how great an influence immunity exerts upon therapy, even though we regard therapeutic innovations with due criticism.

The science of immunity has benefited the clinic, and is now in its turn to be benefited by the clinic. The future belongs to the clinician who, thoroughly embracing the principles of immunity and its teachings, has had at the same time a thorough clinical training in practical work, thus knowing all its requirements and problems. Only this sort of practitioner is qualified to undertake the solution of the great problems which come up in the clinic in the subjects of tuberculosis and syphilis, as well as many questions in acute infectious diseases. Clinical chairs are at present occupied almost exclusively by investigators who combine a knowledge of chemistry with clinical training, and it would be unfair to depreciate the merits of these investigators who have succeeded men who were purely clinicians. Yet they have essentially fulfilled their mission; and the answer to new questions requires a combination of the clinic and immunity research. The progress of internal medicine will be greatly hindered if no suitable positions are provided for clinicians with bacteriological training. The chairs in experimental therapy are at present all connected with theoretical pharmacological institutes, whereas at least a part of them should be connected with clinics. The study of immunity without direct contact with a clinic will likewise lead to errors, and the frequent discrepancies existing between bacteriologists and clinicians are due essentially to a lack of unity between clinic and bacteriology, for the reasons above mentioned. The creation of positions of bacteriologists to the Berlin Municipal Hospital, and the establishment of an institute for experimental therapy at the Eppendorfer Hospital are the first, though for various reasons insufficient, attempts at bridging the gulf between these fields.

The reasons for the many misunderstandings between bacteriologists and clinicians—and in this practitioners

follow, as a rule, the clinicians who have been their leaders in the field of internal medicine—are to be found in the fact that it has been practically impossible for the clinician to familiarize himself with the principles of immunity. The reason for this was that, even among workers in immunity, there were still differences of opinion as to the interpretation of the results of research. These discrepancies, however, are no longer so deep-rooted as to prevent a clear conception of the principal results of research. A science in which no controversy existed would be dead, no longer capable of development, and would hardly afford the internist any incentive for devoting his special attention to it. The chief reason for this misunderstanding is the fact that, until recently, it was very difficult for the physician to keep in touch with the progress of the science of immunity. There is no book which introduces the subject to the general practitioner. We have large textbooks which, even for the expert, are merely works of reference, and we have medium-sized bacteriological textbooks which, of late, treat in part the subject of immunity. There is, however, no book such as is needed by the practitioner to acquaint him with the revolutions going on in medicine, and enable him to read intelligently his journals, that he may not, amidst the cares of his practice, be entirely deprived of the possibility of following the progress of his science. A work which serves this purpose, without the technicalities of bacteriology, is still wanting,* and this small book is an attempt to meet this want.

It is impossible to treat clinical sero-diagnosis alone without taking up the principles of the science of immunity itself. However numerous lectures in scientific associations have been of late, they have not been of value to the majority of practitioners, since it is impossible in such lectures to go into the theoretical premises, and is only possible to report new findings.

* Since the above was written two volumes have appeared: Müller's 'Lectures on Infection and Immunity' (G. Fischer, Jena), and a work by Much (Wurzburg, 1911). The former sets forth a vast number of individual facts; the latter has as its chief aim the making immunity of value to practitioner and clinician.

Infection and Virulence.

The term 'infection' takes us into the very heart of our subject. Infection implies the presence of bacteria with a certain degree of virulence.

The Rôle of Bacteria in Infection.—When during the past thirty years exciters of infection were found in increasing numbers, a too far reaching significance may have been ascribed to these micro-organisms by bacteriologists. The reaction which followed proved that, in spite of the presence of numberless disease producers, the human race had maintained its existence without difficulty. It was at this time that a philosophic brain—that of Rosenbach—invented the phrase: 'Bacteriologists are occupied, not with infection diseases, but injection diseases.'

Essentials of Infection.—However justifiable this reaction may have been in contrast with previous exaggerations, these exaggerations were necessary from an historical point of view. Investigators had been obliged to work with enormous infectious doses in order to ascertain, in an animal material not always adapted, the etiologic significance of individual bacteria as exciters of definite infections. There can no longer be any doubt that two factors are especially concerned in the origin of an infection: First, the virulence of the infecting bacteria, and second, the predisposition of the individual to infection, which may be a *predisposition of the animal species*, a *transitory* or *individual predisposition*. These two factors upon which infection depends are so related that very highly virulent bacteria may produce disease even where a very slight predisposition exists; while, on the other hand, a strong predisposition affords a possibility of successful infection, even with relatively non-virulent bacteria.

But it is only after the invasion of the body by bacteria that the struggle of the protective forces against the invading organism begins—a struggle, the phases of which are evidenced by clinical phenomena, and the features of which we shall study more closely in the chapter on the Theories of Immunity.

We have said of the first factor requisite in infection—viz.,

virulence—that an explanation without hypothesis is not possible. As is known, we speak of virulent and non-virulent bacteria, of an increase and an attenuation of virulence. We may say, perhaps, in a general way that unfavourable external conditions, as, for example, a deviation from the optimum of temperature, lessens virulence; but that the converse does not hold good—*i.e.*, favourable external conditions do not necessarily increase virulence. In order that virulence may increase, a certain selection of bacteria must take place, in which, in the struggle for existence, the less virulent micro-organisms succumb. This takes place in a marked degree in the animal body, and for this reason animal transmission increases virulence to a maximum, and is also the best method of increasing virulence. On the other hand, in a growth of bacteria under luxurious conditions, such as an artificial culture-medium, where the struggle for existence is largely eliminated, no selection takes place, and a rapid loss of virulence follows.

If, as often happens, the disease-producing properties of bacteria are referred to somewhat contemptuously, it should be remembered that these experiments are largely dependent upon laboratory cultures, in which for the most part an entire loss of virulence has occurred. We shall discuss shortly the reasons why animal transmission leads to an increase of virulence by a rational elimination of less virulent bacteria.

Virulence, too, is nothing absolute. In bacteria there is no absolute virulence and no absolute non-virulence—that is to say, under certain external conditions non-virulent bacteria, even saprophytes, may develop virulence; while, on the other hand, virulent bacteria may be overpowered by the protective forces of the body. *Virulence is, therefore, the ability of the bacteria to resist the protective forces of the body and to propagate.* Here we first meet with the term which will continually recur throughout this discussion, 'protective forces of the body.' It is impossible to define bacterial virulence without reference to the protective forces of the body. It becomes evident from this wording of the definition why transmissions (vaccinations) lead to an increase of bacterial virulence in suitable animals only—*i.e.*,

in animals with a certain degree of susceptibility; while in non-susceptible animals, in which bacteria, surviving the struggle with the protective forces, finally cease to propagate, vaccinations lead to a lessening rather than an increase of virulence.

Our therapeutic measures usually aim at immunizing men or animals, more or less successfully, against bacteria and their products. On the other hand, an attempt has been made, not without success, to immunize bacteria against the protective forces of the body, seeking to accustom them to the immune bodies by keeping them in continual contact with immune serum. (*Cf.* Cohn, *Zeitschrift für Hygiene*, vol. xlv., No. 1, and others)

R. Pfeiffer's Theory of Bacterial Virulence.—In order to understand this experiment we must look into the clever theory of virulence held by Pfeiffer and Friedberger, although technical difficulties in experimental work have prevented its positive proof. Nevertheless, this theory promises a clearing-up of the haze of complications surrounding bacterial virulence, and takes us into the heart of Ehrlich's lateral chain theory. According to Pfeiffer, bacteria possesses an apparatus of receptors. If bacteria are to be dissolved, it is only necessary that some of these receptors be occupied by substances of the serum which lead to a solution. The number of receptors varies greatly in individual bacteria; the more receptors a bacterium has, the more protective substances of the serum which effect dissolution of bacteria (so-called immune bodies) it is able to fix. These substances in the serum which are capable of dissolving bacteria, and which we designate as lysins, or, more accurately, as bacteriolysins, are divested of their capacity for dissolving other bacteria by fixation to a bacterium. The more virulent the bacteria the more receptors they possess, and the better able they are to fix the lysins, protecting in this way other bacteria from lysis, and making possible their propagation.

Bacteria which succumb to lysis in a virulent infection play, so to speak, the rôle of a Winkelried, in that they centre the greater part of lytic forces upon themselves, and by

their death make possible an undisturbed growth of the remaining organisms.

The principles of virulence would be exhaustively and irrefutably defined by this theory but for the fact mentioned above, that an absolute proof is still lacking because of difficulties in experimental technic. The proof of the presence of receptor-apparatus is shown by determining the titer of serum—*i.e.*, its content of bacteriolytic immune bodies before the experiment. The bacteria, virulent or non-virulent, are then introduced into the serum, their receptors now affording an opportunity for the bacteriolytic immune bodies to attach themselves to the receptor apparatus. The conditions on which these experiments depended were that if the theory was correct virulent bacteria would fix a greater number of bacteriolytic immune bodies than would non-virulent. The proof was made by centrifugating the bacteria after they had had time to become loaded with bacteriolytic immune bodies, and by testing the serum again for its content of these immune bodies. The difference between this and the first titer indicated the quantity of immune bodies fixed by bacterial receptors. As a matter of fact, bacteria do fix immune bodies, virulent bacteria more than non-virulent; but the differences are not great enough to warrant in themselves an explanation of the remarkable variations in the properties of virulent and non-virulent bacteria.

Virulence Theory of Marx and Woithe.—One other virulence theory should be mentioned. According to this theory, conclusions as to the virulence of bacteria may be drawn from their morphologic properties. This theory treats with the Babes-Ernst corpuscles, well known in diphtheria bacilli—bodies which lie within the bacteria and are well characterized by morphologic and staining properties. The fact that it was possible to detect morphologic differences in the minute bacteria caused at first a great sensation. Discussions as to whether in these granules we had to deal with bodies resembling the cellular nucleus have long since ended, and the view that on this basis might be built a morphologic virulence theory (Marx and Woithe) has not proved tenable. In these Babes-Ernst corpuscles we apparently have to deal with products of secretion, the excretion of which is probably associated with the vital properties, but not directly with virulence. As we have seen, a decided growth and

great capacity for proliferation of bacteria—that is to say, a strong vitality in the usual sense—is not always synonymous with virulence.

Predisposition.—Predisposition is far more difficult to define than virulence. A predisposition is present if the protective forces of the body against a particular infection do not exist in a sufficient degree. This relates usually to quantitative proportions of protective forces. An individual or temporary predisposition is present if the protective forces of the body are impaired or diminished by any cause. That such individual differences occur follows from the well-known fact that even in a severe epidemic only a part of those stricken succumb, while others are severely affected, and still others are only slightly ill. Recent animal experiments have shown that even a vaccination may fail if other bacterial products are injected at the same time—not, of course, in fatal doses. Furthermore, spores of tetanus, harmless in themselves, cause disease after overheating the animal. Cooling or heating renders animals ordinarily immune to tetanus or anthrax susceptible to these infectious diseases. According to Charrin Roger, rats non-susceptible to anthrax can be affected by it through fatigue, which, if we accept Weichardt's theory of toxins of fatigue, may be traced to the simultaneous injection of poisons, together with infectious organisms, as above mentioned.

These observations in animal experiments are of great importance, because they offer an explanation of many clinical phenomena, and because scientific bacteriology and hygiene, by overestimating the factor of infection excitants, had been in apparent contradiction to clinical phenomena. However important it may be to prevent the spreading of infective agents, it is just as important for the individual physician, as well as for social hygiene, *to know the predisposition of people, individually and collectively, to a certain disease*, in order that the disease may be checked. It is an incontrovertible fact that psychical influences, deficient nutrition, alcoholism, unsanitary lodgings, confinements in rapid succession, detention in prisons, and psychical depression favour infection, especially of a tubercular nature. The factor

common to all these apparently different influences is to be found in the general impairment of the organism, in consequence of which the individual cells are offered, thereby reacting upon the protective forces of the body.

The conditions in tuberculosis are extremely interesting. A latent inactive tuberculosis affords a high degree of protection—a protection, however, which has been well-likened to the arsenal of a fort, in which the protective power may at any moment be transformed into destructive force by an explosion. Under the influence of psychical and somatic injuries latent processes may at any time become active, as has been shown by instances in prisons, where the regulations were such that infection from without was out of the question, and where tuberculous foci, inactive in the majority of cases, became active after detention in the prison, for the reason above mentioned.

Another predisposing cause of paramount importance is trauma and mechanical injuries. Bacteria circulating in the organism tend to become localized at points involved by trauma (osteo-myelitis, tuberculosis of the bones, joints, etc.). Such localization, caused by an increased predisposition of tissue, may sometimes be of advantage to the organism. This is shown by a simple experiment. Streptococci injected into the pleural cavity of a rabbit are quickly absorbed, and sepsis arises. If, however, the pleural cavity is previously irritated by an injection of aleuronat the process remains localized in the pleural cavity.

CHAPTER II

Protective forces of the body—Humoral theory of immunity—Metchnikoff's phagocytic theory—Origin of suppurations—Opsonic theory in its relation to phagocytosis—Theory of aggressins.

Protective Forces of the Body.

In the preceding chapter we spoke of the protective forces of the body and the weapons of bacteria as embodied in their receptor apparatus. The powers of bacteria do not end here, but only the weapons of living bacteria. However, virulence does not, as a rule, appear during the life of bacteria, but only after their dissolution. Pfeiffer has applied the apt comparison of a serpent biting and killing after its head has been struck off. The physiologic virulence of bacteria depends chiefly upon poisonous substances (so-called endotoxins) which are freed at the death of the bacteria. Bacteria die or are destroyed in every infection, and for this reason in every infection, whether it leads to death or is overcome by the protective forces of the body, these poisonous substances are liberated by bacteriolysis. Yet the figure used earlier in this volume, likening the bacteria to a Winkelried, is not unfitting. The concentration of lytic substances of the serum upon the self-sacrificed bacteria plays such a part that the other bacteria are enabled to proliferate. So long as the struggle between animal and bacteria is undecided, the proliferation of bacteria and their lysis is approximately the same, and only after the struggle has ended in favour of the bacteria does a luxuriant proliferation take place. Death in infections was long regarded as due to mechanical displacement caused by this proliferation, as, for example, in anthrax.

As a matter of fact, death is caused not by living, but by dissolved, bacteria. An active proliferation of bacteria is unfavourable only for the reason that the micro-organisms cannot be removed except by lysis, and because a strong proliferation gives rise to the danger of large quantities of poison being liberated by lysis—quantities too large to permit of a continuation of life.

Bacteriolysis in Infection.—The present stage of our knowledge can be summarized in these two sentences, based on Pfeiffer's teachings. Radziewsky asserted that a bacteriolysis also takes place in virulent infections. Wolff-Eisner discarded the 'also,' and formed the sentence thus: '*Only by a dissolution of the bacteria can death occur at all.*' The proof of this may be briefly mentioned. In animal experiments there are, generally speaking, two results possible: either the animal becomes free from bacteria, or it dies after the bacteria have actively increased. Between these two extremes there is an intermediate condition, in which the animal dies, although all bacteria have been destroyed. This is the noteworthy case of the so-called *sterile death*, which can scarcely be explained except by the assumption that the animal has combated the bacterial infection by means of its protective forces, but that so much poisonous substance has been freed by the bacteriolysis that the minimal dose has been exceeded. Experiments with injections or more particularly reinjections of dead bacterial bodies, by which it is likewise possible to cause death without an increase of bacteria, may be cited as further arguments for the proof of this assertion. Wolff-Eisner ascribes this action of the bacteria to the fact that in those poisonous substances of the bacteria we have to deal with a general poisoning by heterogeneous albuminous substances. The poisonous action varies with individual bacteria, he proved, just as enormous quantitative differences exist in the toxicity of individual kinds of albumen. Thus, for example, the toxicity of serum albumen is to that of sperma about as the toxicity of the typhoid bacillus is to that of a saprophyte. Since, therefore, the action of bacteria must be considered in accordance with the great fundamental law of the toxicity of heterogeneous albumen, there is no

absolute virulence and no absolute non-virulence. Therefore virulence must remain a relative term, because special conditions permitting an undisturbed propagation of a saprophyte may, in the dissolution of this relatively non-virulent bacillary albumen, allow it to act in such quantities that it has a poisonous effect similar to that of the relatively non-poisonous serum albumen.

The fact that the action of the great majority of bacteria becomes manifest only after their dissolution, has been accepted only after a long time, and with much hesitancy, by the students of immunity, and still more unwillingly by the clinician. Seeing, on the one hand, an increase of bacteria in fatal cases, and on the other a destruction of bacteria in convalescent cases, it was difficult for physicians to understand that the manifest increase of bacteria was playing only an indirect part in the outcome, and that consequently the lysis of bacteria, being, as it were, concealed, had to play the determining rôle. Eisenberg has recently modified this theory somewhat, stating that poisonous substances were liberated, not only by bacteriolysis, but that viable, proliferating, undissolved bacteria were capable of excreting poisonous substances. The simplest method of picturing this process, and one in full accord with our knowledge of endotoxins, is to assume that bacteria excrete small fragments of protoplasm. This theory will undoubtedly satisfy the clinician from an etiologic standpoint, since it enables him to ascribe an active rôle to the bacteria propagating before his very eyes ; and the subject of endotoxins in the old or a new form will gain admission to the clinic more readily than before. In this sense the modification is to be welcomed. However, it must be emphasized that this modification does not necessitate any essential difference. As has long been known, there is a so-called law of everlastingness of bacterial protoplasm. While in more highly organized animals only one cell of the whole complex is concerned in propagation, bacterial protoplasm is continually subdividing, the new cell consisting essentially of the protoplasm of the original cells. It is therefore irrelevant, so far as the principle is concerned, whether we assume that one bacterial cell is divided into two

bacteria, that one cell is dissolved, and the other continues to propagate, etc., or whether we take for granted that the living cell casts off protoplasm—*i.e.*, is divided—one product of division being immediately dissolved.

So far we have only said that the protective forces cause the destruction of bacteria by lysis. As we have seen, poisonous substances are liberated by lysis of bacteria, and the lytic protective forces of the body therefore act favourably only when they succeed in destroying the bacteria so quickly that no considerable amount of poisonous substance is liberated by their lysis. This is one of the chief facts of immunity, and it offers an explanation for innumerable clinical and experimental observations. Yet we must not underestimate the significance of these lytic protective forces, by means of which we defend ourselves daily against invasions of bacteria or destroy invading bacteria so rapidly that the infection is not even brought to our notice by the appearance of disease symptoms. Besides these humoral protective forces, however, there are also cellular forces located in the leucocytes. The advocates of the different views are still contending vigorously for first rank, and up to a short time ago this subject was still the chief bone of contention in the question of immunity. Although these differences are probably overestimated, we shall discuss the question more at length later on in this work.

Antitoxic Protective Substances in the Serum.—The protective forces residing in the serum are also manifold. An agreement has not yet been reached as to the significance of the individual components of these protective substances. We have spoken of the one chief protective substance, the bacteriolysins. We shall now mention a second important group directly opposed to the action of poisons, the antitoxins, which differ essentially from lysins. Antitoxins are of interest from an historical view-point, for the reason that the study of the entire science of immunity originated in them. The number of toxin-producing bacteria is, however, extremely small in comparison with the total number of pathogenic bacteria.* Antitoxins neutralize the poisonous

* Diphtheria, tetanus, anthrax, and botulismus bacilli are the only generators of toxin. Kruse ('Handbuch der Serumtherapie,' Lehmann, München,

substances *secreted* by the bacteria. It has not yet been ascertained what becomes of the toxin-forming bacteria after their toxins have been neutralized. However, this much is certain, toxin-forming bacteria cannot exist if the body is able to neutralize their toxins with antitoxins. Under such circumstances they are destroyed by lysis before producing disease symptoms; they are phagocytized by leucocytes, or, as has been noted particularly in diphtheria, they remain for a long time unaltered at the same spot, without eliciting signs of disease (bacilli carriers). Aside from such healthy persons carrying diphtheria bacilli, there are abundant instances of typhoid bacilli carriers.

These bacilli carriers are of great significance in spreading diseases and epidemics. They are now known to have a great deal to do with the origin of endemics of diphtheria and typhoid.

To the individual affected, this condition is generally of no importance. The bacteria live in his organism without producing disease symptoms. Yet no symbiosis occurs between bacteria and body juices such as is observed, for example, in trypanosoma diseases, where trypanosoma may live in the blood in greater or less numbers without the production of disease symptoms. Bacteria are apparently unable to live in the blood in symbiosis without creating disease symptoms.

The bacteria of bacilli carriers live, like the colon bacilli of the intestine, in parts of the body where they do not come in contact with the serum or blood (absence of contact reaction, which, according to my views, is necessary to the formation of antibodies, but is also requisite for the production of disease symptoms). If a contact arises, even coli bacilli may produce severe disease symptoms (colisepsis), and even typhoid bacilli carriers may become affected by typhoid or suffer a typhoid relapse.

1910), contrary to Dörr, does not consider the bacillus of dysentery a toxin producer.

Humoral Theory of Immunity.

For years there was a struggle between the humoral and phagocytic theories of immunity, and even yet this warlike attitude continues, even though the differences between the two theories are no longer so deep-rooted, excepting, of course, for many dissimilarities in details. For though the phagocytic theory assumes the seat of immunity to be in the white blood-cells, the humoral theory of serum action regards the cells of the hæmatopoietic organs (the mother cells of the white blood-corpuscles) as the chief source of immune bodies, which circulate in the serum.

The serum, circulating through all organs, is well adapted as a means of communication to transmit immunity against any infecting organism to all stations, even the outposts. The phagocytes, to be sure, possess the same property, and, possessing motility, and circulating in the serum, they are able to reach all parts of the body to which the serum permeates.

Thus, serum and phagocytes are in reality the only elements which by virtue of this property—common to both—may be taken into consideration.

In this respect the individual cells of the organs do not seem to possess any function. This would be of significance only if all cells possessed this inherent property independent of the organic differentiation. But the organs play a very important part in neutralizing poisons of toxins as well as endotoxins, as the author has recently shown (*Zentr. f. Bakt.*, vol. xlvii., Nos. 1, 2). Practically no attention has yet been paid to this cellular immunity. There can be no doubt, however, that such organ immunity for neutralizing poisons really exists, and it will be the task of future investigators to throw light on the subject.

Metchnikoff's Phagocytic Theory.

In order to properly understand Metchnikoff's phagocytic theory, a brief survey of the field of hæmatology is necessary.

Metchnikoff, as we know, differentiates two kinds of leucocytes, generally speaking, macrophages and microphages. Microphages possess the property of destroying bacteria,

while macrophages destroy dying body cells or heterogeneous cells as well as protozoa which have found their way into the body. Apart from the fact that the separation of protozoa from bacteria involves some difficulties, simple experiments show that macrophages may also take part in the ingestion of bacteria, particularly tubercle bacilli. Even in Metchnikoff's system this separation cannot be strictly carried out, since to macrophages, lymphocytes particularly, a leading rôle is assigned to tuberculosis immunity.

It is interesting to note that Ehrlich, too, has classified leucocyte types as leucocytes and lymphocytes, granulated and non-granulated cells. This classification was very nearly analogous to that of Metchnikoff, agreeing with it in the most essential points, and it was like that of Metchnikoff in that finally this close differentiation was not maintained. Ehrlich gave three reasons for the differentiation of these two types of leucocytes which he regarded as the summary of many years of study.*

1. Leucocytes are mobile; lymphocytes immobile.
2. Leucocytes are granular; lymphocytes non-granular.
3. Leucocytes have power to react to chemotactic stimuli; lymphocytes have not. Where there was an increase of lymphocytes in the blood it could only be explained by mechanical flushing.

This essential distinction between lymphocytes and leucocytes could in no way be maintained. Hirschfeld and Wolff-Eisner have established the motility of lymphocytes. By means of the lymphocytic exudates Wolff-Eisner has shown that leucocytes follow actively chemotactic stimuli, and Michaelis and Wolff-Eisner have also found a granulation in lymphocytes—the Azur granulation. (*Cf.* Pappenheim, Schridde, Jagic, and others.)

The undoubted differences between the two types of cells is a difference in function. In all probability both kinds of cells originate from the same form, the lymphoid cell, which has retained its capacity for forming cells of a different type in cases of necessity. This theory conforms with the em-

* Ehrlich's conclusions, Nothnagel's 'Anæmia, Pathology and Therapy,' vol. viii., part 1, No. 3.

bryological, comparative-anatomical, and clinical observations already known—lymphoid cell leucæmia. (*Cf. Zeits. f. Klin. Med.*, vol. xlv., Nos. 5, 6).

Metchnikoff's microphages* correspond to Ehrlich's leucocytes. The macrophages correspond roughly to Ehrlich's large lymphocytes. In addition to these, there are mononuclear leucocytes (Ehrlich's so-called large mononuclears) and other blood cells, notably the pulp cells of the spleen. The small lymphocytes, ranking first among the white blood cells in number and significance, can in no way be classified in Metchnikoff's scheme. On the whole, however, the phagocytic theory conforms to Ehrlich's original scheme—*i.e.*, in the fundamental differentiation of leucocytes (in a narrower sense, the polynuclear neutrophile granular cells, which alone are supposed to play a part in the struggle with bacteria) from the mononuclear leucocytes, which are supposed to phagocytize protozoa and body cells. Inasmuch as recent investigations have undermined Ehrlich's close differentiation of leucocytes, the base of Metchnikoff's morphologic differentiation of macrophages and microphages is shaken, so far as anatomic and physiologic proof—motility—is concerned.

As a rule, blood and organs in a healthy animal are free from micro-organisms. The migration of bacteria into the blood immediately after death indicates that bacteria entering the blood during life must be immediately destroyed. French authors have stated repeatedly that bacteria are to be found in the blood during life, but these individual findings, in so far as not being attributable to a faulty technique, merely help to prove, when taken in connection with this sterile condition of the blood, that bacteria constantly entering the blood must be destroyed, and that the body has the power and the tendency to keep the blood constantly sterile. This is confirmed by the fact that blood which has harboured bacteria during an infectious disease becomes entirely free from bacteria *after recovery*. In the same way pleuritic and arthritic effusions containing bacteria often become sterile,

* Metchnikoff, 'L'Immunité dans les Maladies Infectieuses,' Paris, 1901.

and often in so short a time that difficulties may arise in the bacteriological diagnosis.

This ability of the body to destroy bacteria has attracted the attention of investigators, and all attempts at building up a theory of immunity have started from this point. For example, it has been determined that forty-eight hours after putrid substances were injected into rabbits the blood had become sterile, provided the animal withstood the infection. Fodor and Wyssokowitsch, in 1885, instead of putrid mixtures, experimented with pure cultures, and were able to prove that many millions of bacteria were killed in the body of the animal. The sterility of the animal's body is, however, only apparent, and confined to the blood in actual circulation; for bacteria pass into the filtration apparatus, such as the spleen, bone-marrow, etc., and are retained there, as Ernst Cohn has proved of argentum, Credé and Wolff-Eisner of toxins (*Zentr. f. Bakt.*, vol. xlvii., No. 10; *ibid.*, Nos. 1, 2). Even in cases in which the infection afterwards prevails the bacteria at first disappear from the blood. This fact is explained by retention of the bacteria in the organs of filtration, for the question of the destruction of the bacteria is not decided when the bacteria disappear and the blood becomes sterile, but depends upon whether the bacteria in the filtration apparatus have also been destroyed.

Bacteria which have invaded the body are not destroyed by their own secretions. If the infection is overcome, they must necessarily have been destroyed *by the body itself*.

To what this destruction of bacteria is due is a problem that still occupies investigators, who are divided into two parties, the one holding that the bacteria are destroyed by *cells*, the other by the *power of serum*.

Historical Outlines of Immunity Research.—The first theories of immunity assumed a humoral direction. In 1887 Fodor found that the blood of rabbits acted destructively upon bacteria. To prove this bactericidal power he applied the plate method. Fisher and Baumgartner raised the objection that with this method bacteria were transplanted from serum upon the culture medium, and thus a

plasmolysis might stimulate a bactericidal action, the former being due merely to isotonic concentration of the new culture medium.

In 1888 Flugge and Nuttall demonstrated that a destruction of anthrax bacteria could be observed in hanging drops by applying the blood-serum of a chicken, frog, pigeon or sheep; further, that bacteria exposed to the action of the serum showed first poor staining properties, and secondly granular destruction. These authors also demonstrated that cholera bacilli are first dissolved for two or three hours in fresh, active, immune serum, and that not until then does an increase of bacteria take place.

Buchner determined that a temperature of 55° C. was sufficient to destroy the bactericidal power of serum. This was a fundamental discovery, being the forerunner of Ehrlich's separation of protective substances of serum into immune bodies (amboceptors) and complement (Buchner's alexin theory). This theory met with the objection from Behring and Nissen that the destruction of bacteria as observed in the plate method or under the microscope did not run parallel with the resistance which the animals showed against infection; that, for example, animals with a strong bactericidal power of serum were sensitive to infection, and *vice versa*. This may be illustrated by an example. The blood of the rat is highly bactericidal, yet rats are susceptible to anthrax infection. Dog's blood is but slightly active against anthrax bacilli, yet dogs are very difficult to infect with anthrax (Behring, *Deut. Med. Woch.*, 1903, 1904).

This was the state of affairs when Metchnikoff's phagocytic theory appeared. This theory is still very widely known. It should be kept in mind that there are three very different views of this theory, each reflecting modifications by which the theory was supposed to be reconciled to facts discovered later. The phagocytic theory is unquestionably a cellular theory. However, to regard it as vital in contrast to the humoral theory appears incorrect, because the humoral theory is essentially of a vital nature.

The original conception of Metchnikoff's phagocytic theory was

as follows : The leucocytes are specific 'fighting cells.' Just as amœba ingest bacteria for nutritive purposes, the leucocytes consume dead tissue intended for resorption, together with living bacteria. If the leucocytes are successful in ingesting all the bacteria, they are victorious, and their victory means the overcoming of the infection. If, on the contrary, the leucocytes succumb in the struggle, if they are destroyed by the poisons excreted by the bacteria, the bacteria are victors, and the animal is overcome by the infection.

The proofs submitted by Metchnikoff in support of his first conception of the theory were, generally speaking, as follows :

1. The schizomycetic disease of Daphnia, of little interest to physicians.

2. The fact that spirilla of febris recurrens disappear in the phagocytes of the spleen as the attack approaches its end.

3. An example found in erysipelas, in which disease leucocytes are found without streptococci in the centre, where healing takes place, while streptococci appear at the periphery, where the disease spreads, without opposition from the leucocytes.

Objections of Baumgarten, Weigert, etc.—The bitterest antagonist of this theory was Baumgarten, who placed his own theory against that of Metchnikoff—viz., that leucocytes are not fighting cells, but 'grave-diggers,' which devour dead bacilli only; that at most they are to be regarded merely as scavengers of the battle-field. Metchnikoff replied to Baumgarten that anthrax bacilli within the leucocytes of the frog grow to long threads, and are able to rupture the cell. This reply to Baumgarten's argument was very striking, but Metchnikoff showed at the same time that the battle was by no means decided by the ingestion of bacteria by the leucocytes, as his original theory assumed. For example, in tuberculosis the leucocytes may become direct disseminators of infection (Klebs and other authors).

Weigert likewise opposed the first version of the phagocytic theory, using as an argument Metchnikoff's proof, deduced from the relapsing affection. It appeared to him

inconceivable that leucocytes would not devour the spirilla while few in number, but would wait, as Metchnikoff's theory had it, until the spirilla had greatly increased. With the remarkable ingenuity peculiar to this investigator, he declared that there must be other forces enabling the leucocytes to enter the battle at this particular moment. Moreover, in relapsing fever and malaria the ingestion of the specific micro-organism does not prevent a new outbreak of infection after an interval, often with increased violence. Consequently, we cannot consider the disease terminated simply because a phagocytosis of the specific organism has been determined at that time.

It was further objected to the phagocytic theory that phagocytosis in gonorrhœa bore no relation to the healing tendency, and also that phagocytosis of the tubercle bacilli by giant cells was not, so far as was known, conducive to recovery, but led to a constant destruction of cells.

Metchnikoff now argued, on his part, that frogs lost their immunity against anthrax at 37° C. and that bacteria enclosed in sacs of collodium are able to grow, even in an animal body insensitive to infection. These two observations, although very interesting, proved nothing either for or against the phagocytic theory, for at a temperature of 37° C., not only the vitality of leucocytes, but that of all other cells, is impaired, and bacteria in a collodium sac are protected against the non-dialyzing immune bodies of the serum, as well as against the action of the leucocytes.

Another series of facts necessitated a modification of the theory. For instance, it was ascertained that leucocytes of guinea-pigs phagocytize non-virulent anthrax bacilli, while not consuming virulent anthrax bacilli. In immunized animals, however, there was no difference in the action of leucocytes toward virulent or non-virulent bacilli. Metchnikoff's second view can, therefore, be expressed in the following way:

Second Version of the Phagocytic Theory; Positive and Negative Chemotaxis.—*There is a positive and a negative chemotaxis of leucocytes. Virulent bacteria act negatively chemotactic; non-virulent bacteria show a positive chemotaxis against*

*leucocytes. Immunity depends mainly upon phagocytosis. Besides this, there is an extracellular lysis.**

But even this version of Metchnikoff's theory can by no means be maintained, for, in spite of positive chemotaxis, death of the animal quite frequently occurs. An example of this is found in septicæmia of mice, according to Verigo; also in my own experiments in intravenous infections of rabbits with anthrax; sometimes also in cholera and typhoid infections. In injections of toxins a fatal termination is very constant, in spite of positive chemotaxis—a fact which Metchnikoff, as we know, also wished to imply in his phagocytic theory. The phagocytes are frequently influenced in a positive chemotactic manner by toxins up to 100 times the fatal dose, without the fatal termination being in the least influenced thereby.

The experiments of Richard Pfeiffer and his school (Kolle, Marx, Radziewski, Ascher, Friedberger, A. Wolff-Eisner, and others) bore evidence that, besides phagocytosis, there is an extracellular dissolution of bacteria. Metchnikoff's theory had to compromise with this, and, consequently, there followed a third version of the theory, viz.:

Third Version of the Phagocytic Theory.—*Phagocytosis is the most important phenomenon in the origin of immunity. The phenomena of immunity are directly or indirectly dependent upon leucocytes. Leucocytes are builders of immune bodies and of the complement; hence even extracellular lysis can be referred definitely to leucocytes.*

Viewed in this light, the cellular and humoral theories were no longer incompatible, for the advocates of the

* It may be remarked here that the hyaline border of bacteria is regarded by Metchnikoff as a toxic border, possessing negative chemotaxis. It is certain that we have to deal with a defensive organ of bacteria, not an aggressive one. Nuttall demonstrated that even bacteria with such a toxic margin could be destroyed. Metchnikoff replied that these bacteria had been phagocytized once, but had been expelled on account of their toxicity; so even these bacteria could not be destroyed without the action of leucocytes. We are reminded here of the findings of Radziewski (*Zeits. f. Hyg.*, vol. xxxvii.), according to which destruction of bacteria is observed even in the most virulent infection. This observation was modified and amplified by Wolff-Eisner (*Berl. Klin. Woch.*, 1903, Nos. 17-20), in that in even the most virulent infection not only were bacteria destroyed, but that death was caused only by the lysis of bacteria.

humoral theory also assumed that immune bodies and their complements were eventually secreted by cells, and in one instance—typhoid fever—Pfeiffer and Marx have even shown that a production of immune bodies may take place in the hæmatogenic organs (*Zeits. f. Hyg.*, vol. xxvii., 1898). It is of little importance whether or not we assume that fully developed forms of leucocytes, as well as young forms, may produce immune bodies.

Struggle for the Third Version of the Phagocytic Theory.—Nevertheless, Metchnikoff carried on a bitter struggle for the third form of his theory. For, according to his view, leucocytes do not actually secrete the complement, but only lose it when destroyed by leucolysis (phagolysis). An extracellular destruction of bacteria should, therefore, be possible only after the leucocytes have been destroyed. The phenomena of the so-called resistance experiment* can be explained in a similar manner. Here phagocytosis is said tc be due to the leucocytes having been made resistant to lysis by preceding injections of bouillon into the peritoneum.

This attempt to maintain the phagocytic theory in its strictest sense is evidently somewhat forced, for a destruction of leucocytes is constantly going on in the animal body. Hence, according to Metchnikoff's theory, complements are constantly circulating in the serum, and, because of this very presence of the complement in the serum, leucolysis, has nothing to do with phagocytosis. Metchnikoff assumes that the phagocytosis, recognizable in resistance phenomena, is due to the resistance of the leucocytes to leucolysis following an injection, and therefore an extracellular bacteriolysis is not observed. However, one may easily convince himself that extracellular bacteriolysis takes place also in

* Under resistance phenomena the following is understood: After a peritoneal infection of sterile liquid (bouillon, salt solution, etc., also nucleinic acid solution), an aseptic inflammation and an exudate occur in the peritoneum. So long as this inflammation persists there is an increased local resistance to bacterial infection, limited to the peritoneum. I have ascribed this resistance to the activation of the protective forces of the body, and have attributed the action of Bier's congestion to the phenomenon of resistance (*Münch. Med. Woch.*, 1906, No. 23). The school of Miculicz has recently successfully applied the phenomenon of resistance to surgery by injecting nucleinic acid sodium twenty to twenty-four hours before operation.

resistance experiments (Pfeiffer); moreover, the author was able to prove with a vital stain that a resistance of leucocytes did not exist here, if anywhere, and that leucolysis was present in an extreme degree.

Metchnikoff furthermore tried to support his theory by very complicated experiments. He believed he was able to show that there was no complement present in the plasma of the blood, but that complement was present in blood-serum—that is to say, in the absence of a destruction of leucocytes in the plasma the complement is absent. It has not even yet been proved positively that the leucocytes represent to a certainty the location of the formation of complement (Ascher and Schneider, *Arch. f. Hyg.*, 1909). That is why this part of the theory is now universally rejected.

Results of the Struggle for the Phagocytic Theory.—After this survey of Metchnikoff's theory, we may sum up the present views as follows: Bacteria are taken up by leucocytes, this process surely being of some significance. Besides this, extracellular lysis unquestionably plays an important rôle. Extracellular lysis is brought about by the co-operation of two substances—viz., lytic immune bodies, or amboceptors, and the complement. These substances are secreted by the cells of the body—*e.g.*, young forms of leucocytes in the hæmatogenic organs. It may even be possible that fully-developed leucocytes participate in the production of these substances.

A greater significance is ascribed to extracellular lysis than would seem justified by direct observation of extracellular bacteriolysis. While bacteria ingested by leucocytes are very easily shown by staining methods, bacteria having undergone lysis escape our observation, because when dissolved they are no longer stainable. From observations in infectious diseases, particularly advanced tuberculosis, we cannot but conclude that the lysis of bacteria is of great significance, this lysis being recognized by its effects, although in the case of tubercle bacilli it almost escapes our vision. (*Cf.* remarks on p. 103).

Links between the Humoral and Phagocytic Theories.—From these explanations it would appear that no incompat ble differences need exist between the phagocytic and

chemical theories. It would be useless to deny that phagocytosis is of importance in the processes of immunization. In this respect it is interesting to note that Wolff-Eisner, in a publication from Pfeiffer's Institute, has taken the first steps toward bridging the gap between these two views, which were regarded vastly different (*Berlin. Klin. Woch.*, 1903, Nos. 17-20). It can be proved with the aid of stains that substances ingested by phagocytes undergo oxidative processes within the leucocytes. It is further known that a formation of anti-endotoxin takes place in but a very slight degree, if at all, in the serum, and, finally, that most endotoxins, even toxins, are very sensitive to oxidative processes. The ingestion of bacteria by leucocytes is thus the only known method of binding endotoxins to stable tissue receptors, and neutralizing by affinity the damaging effect of endotoxins upon the body.

After these observations, we cannot but ask where the fascination lies which Metchnikoff's theory in its original form still holds for large circles of people.

It should be mentioned in this connection that even to-day works on research are published supporting the original form of Metchnikoff's theory — a position which Metchnikoff himself gave up as untenable many years ago. In future publications the three different versions which this theory have undergone in the course of time should be sharply differentiated.

These repeated references to the original version of Metchnikoff's theory are due to the fact that in all experimental as well as natural infections those cases in which a strong phagocytosis is determined inevitably tend toward a favourable termination. As we shall see in discussing bacteriolysins and opsonins, phagocytosis occurs whenever bacteria have been sensibilized—*i.e.*, saturated with immune bodies, such as opsonins. Weigert had already anticipated this (see above).

As we see, further, the fate of the animal is not decided by the content of bacteriolysins alone, but is dependent upon other factors. Yet, everything being equal, the content of lysins is of advantage, and consequently a strong phago-

cytosis, being an indicator of the presence of sensibilizing immune bodies, must be regarded with a grain of salt as the sign of a favourable termination. It requires a considerable amount of the spirit of criticism to rid oneself from this constantly renewed impression. The reason for the predominance of phagocytosis in infections running a favourable course is founded upon a simple biological law (Pfeiffer's law of positive and negative chemotaxis), which applies as well to bacteria—viz., 'A chemotactically active substance in a certain degree of concentration acts upon leucocytes in a positive chemotactic manner. If the concentration exceeds a certain degree, the opposite effect—*i.e.*, a negative chemotaxis—is produced.'

It can easily be determined that small amounts of heterogeneous albuminous substances, such as small amounts of endotoxin originating from bacteria, act in a positive chemotactic manner, while large amounts act in a negative chemotactic manner. Since cases in which only small amounts of endotoxins have been liberated very often recover, and cases in which endotoxins have been freed in large amounts usually succumb, one might easily form the idea that the presence of phagocytosis and recovery from infection are related. It requires a great deal of critical experimental work to find the intermediate cases that cannot be classified in this scheme. Take, for example, the so-called sterile death in typhoid and cholera infections with positive phagocytosis. Here is a case in which all the bacteria have been dissolved by the bactericidal agents of the body, yet endotoxin concentration has not yet passed the point where positive chemotaxis is converted into a negative one, and in which, nevertheless, the amount of endotoxin liberated by bacteriolysis has sufficed to cause death. For instance, it is not at all uncommon to find cases, especially in paratyphoid, in which, in spite of a marked phagocytosis, death occurs in test animals with an abundance of bacteria in the peritoneum. Such cases flatly contradict the second version of Metchnikoff's theory—the favourable action of a positive chemotaxis upon the termination of the infection.

Summary of the Present Knowledge of Phagocytosis.—A brief summary of our present knowledge of phagocytosis is as follows:

1. Leucocytes consume bacteria when in their vicinity—especially, then, in the so-called resistance experiment.

2. Phagocytosis is increased if the bacteria are sensibilized —*i.e.*, saturated with amboceptors (immune bodies, or opsonins).

3. For this reason a strong phagocytosis is an indicator of the presence of quantities of amboceptors (bacteriolysins).

4. Since, as a rule, the presence of bacteriolysins is advantageous in overcoming an infection, phagocytosis is an indicator of a course of infection with a favourable termination. To regard phagocytosis alone as responsible for overcoming infection is to confuse the indicator with the principal cause.

5. That infections with strong phagocytosis do not in all cases run a favourable course, as in paratyphoid or tuberculosis, is due partly to the fact that the presence of bacteriolysins is not synonymous with the overcoming of an infection, and, further, that the fatal dose of bacterial poison and the dose acting as a negative chemotaxis do not correspond. Thus, fatal infections occur in spite of positive chemotaxis in typhoid, cholera, tuberculosis, and more particularly in paratyphoid.

6. Even under these conditions the leucocytes have assigned to them the important function of destroying endotoxins, probably by oxidative processes, a property causing the significance of leucocytes in the struggle with infection to appear very great, since other anti-endotoxic forces are available, if at all, only in very small quantities.

7. It has not been proved that complement is formed by the destruction of leucocytes. However, if complement should originate in this way, the blood-serum circulating in the body would always contain free complement, because the serum is the conveyer of substances resulting from broken-down leucocytes.

The Origin of Suppurations.

In connection with the theory of phagocytosis, we shall describe briefly the origin of suppurations.

By 'exciters of suppuration' very definite bacteria, staphylococci and streptococci, are understood. This conception, however, is misleading, since every heterogeneous

reabsorbable albumen may, in proper concentration, create a suppuration. A positive chemotactic suppurative action occurs in bacterial infection only when bacteria are destroyed by lysis. Thus, the appearance of pus is indirectly a sign of a destruction of bacteria, and in this sense is not always an unfavourable symptom, as has often been observed clinically, inasmuch as suppuration prevents sepsis by localizing the disease focus.

The Opsonic Theory in its Relation to Phagocytosis.

The definition and technique of opsonic experiments and their diagnostic, prognostic, and therapeutic significance will be discussed in detail in a separate chapter (see p. 116). We are concerned here only with the fact that opsonic experiments are often regarded as proof of the correctness of the phagocytic theory, for the simple reason that in opsonic tests the bacteria ingested by leucocytes are counted. This view is, however, entirely erroneous, for in these very opsonic experiments it is unmistakable that the phagocytosis accomplished by leucocytes without a serum (so-called spontaneous phagocytosis) is practically nil. It is shown, further, that in applying serum which is not entirely fresh to induce phagocytosis, complement has to be added, as the complements that are supposed by Metchnikoff himself to be in the leucocytes do not suffice. Certainly in opsonic work the necessity is soon apparent for considering both factors, and combining phagocytosis with the humoral theory. The experiments of Wolfsohn and Wolff-Eisner have shown that in opsonic tests the rôle of the leucocytes cannot be neglected, for even with the same serum the phagocytic power of leucæmic leucocytes was weaker than that of the normal, and that of guinea-pig leucocytes less than that of human.* The statements concerning leucæmic leucocytes have been confirmed by experiments of French authors.

* These last experiments refer only to opsonizing tubercle bacilli.

Theory of Aggressins.*

The theory of aggressins was constructed by Bail and his school, and promised for some time to revolutionize the theories of immunity and immunization therapy. Bail assumed that bacteria produce specific aggressive substances, which cripple the protective forces of the body. Immunization against bacteria could, indeed, be brought about with such aggressins, and, according to Bail's experiments, the following properties were ascribed to them :

Aggressins (obtained by filtration of bacterial exudates through Chamberland filters), when injected together with bacteria, are able to make sublethal infections lethal, and non-virulent bacteria virulent. Moreover, if injected together with bacteria, they act upon leucocytes in a negative chemotactic manner. In this respect the aggressin theory is related to the phagocytic theory, for paralysis of the protective powers of the body by the aggressins was regarded as due to keeping the leucocytes at a distance.

Aggressins and their Relation to Endotoxins.—All these properties of aggressins can be explained by assuming that bacterial exudates necessarily contain dissolved bacterial substances (endotoxins). These endotoxins are added to the endotoxins originating from the infection, and the fatal dose is thus reached earlier than it would otherwise be. It is simply a question of addition, explaining at the same time the specificity of the so-called aggressins, and also their negative chemotactic action, the latter being exerted, not by the aggressins alone, but *only* in contact with homologous bacteria. It can easily be deduced from the foregoing remarks that negative chemotaxis, particularly in intermediate cases, cannot be a characteristic of aggressins. In fact, the author succeeded in observing positive chemotaxis in aggressin experiments also. It may even happen that an aggressin exudate can contain free bacteriolysin as well as endotoxins. Such an aggressin may develop a considerable endotoxic and aggressive action, owing to the double action

* Exhaustive literature in Sauerbeck: 'Neue Tatsachen und Theorien in der Immunitätsforschung,' Wiesbaden, 1907.

of the endotoxin and the bacteriolysin, which liberates new endotoxins from the bacteria (from unpublished experiments).

The experiments of Wassermann's school showed that we can also obtain *in vitro*, by extracting bacteria, liquids possessing all the properties of aggressins, and it was proved by this that in aggressin experiments we had to deal with the addition of toxic substances of bacterial endotoxin to substances liberated from the injected bacteria.

The aggressin theory cannot be considered a special theory in immunity, for the reason that the aggressin is not a specific substance secreted by bacteria and heretofore unknown. Nevertheless, the wearisome and painstaking work of Bail and his school has not been in vain. They have greatly amplified our knowledge of endotoxins and their action, and have more especially enabled us to find a form of endotoxins which permits experiments even with such unstable substances as active solutions of endotoxins.

CHAPTER III

Ehrlich's lateral-chain theory—Further conclusions derived from this theory—Summary.

Ehrlich's Lateral-Chain Theory.

EHRLICH'S lateral-chain theory blazed the trail for immunity research. There has been much discussion as to whether this theory was entitled to be considered dogmatic; indeed, a distinction has been drawn between the advocates of this theory, according to their various degrees of devotion to it. He who makes such a distinction neither does justice to the spirit of the theory nor to the genius of its originator, for Ehrlich's lateral-chain theory is a figure symbolizing our ideas of immunity, and making it easy to popularize the subject. Its greatest value, however, consisted in placing a groundwork of theory under new problems, which could then be solved by experimentation. In this sense friends and foes of this theory are alike indebted to it.

The fact which should probably be taken as a starting-point for this theory is that a body may become insensitive to certain poisons, known as toxins, and that the body juices (serum) of such an individual are capable of protecting a second individual against the action of this poison. A person may become insensitive to chemical poisons, as morphine and many others, but this insensitiveness cannot be transferred through the serum to another individual. In the latter case it is probably correct to assume that toleration is established, whereby the cells become accustomed to living in the body juices (see Chapter IV., on Hypersensitiveness).

Concerning transmissibility of insensitiveness to toxins,

Ehrlich's theory assumes the following conditions, fully explaining thereby all that has been observed on the subject:

Toxins and Toxoids.—Toxin, according to Ehrlich, is a complex of molecules possessing a toxic group (toxophores) and a haptophore group, capable of union with the cell, thus bringing the toxic group into its immediate vicinity.

The conclusions confirmed by facts to be drawn from this theory are as follows: If the haptophore group does not fit analogous cell groups (cellular receptors), a toxic action cannot take place. The same is true if the haptophore group of the toxin has been occupied in the serum, since toxic action can occur only through the union of the haptophore group with the cell receptors. If a destruction of the toxophore group alone occurs, toxic action is also precluded. This constitutes the so-called toxoid formation.

If the toxin, through its haptophore group, joins a cell receptor, the toxic group is now able to act upon the cell, after which two results are possible—either the toxic effect is so great that the cell is destroyed, or the cell is able to defend itself against this effect by casting off the receptor to which the toxin is fixed. Further action of the poison on the cell is thereby prevented, and the cell can now repair the damage. The elimination of the poison has not been accomplished without incurring a defect: the cell has lost its receptor, which was the point of assault of the toxin. Since the body of the higher organism is able to repair defects, a re-formation takes place, even to a greater extent than a simple replacement of the loss (Weigert's biogenetic law). In this case the lost cell receptor is replaced in excess. The newly-formed receptors do not all adhere to the cell, but some are cast off into the serum. These are theoretical constructions, but they illustrate in a very realistic manner the conditions observed in the effect of toxins and the formation of antitoxins.

Further Conclusions from this Theory.

Antitoxins.—If such receptors are circulating in the serum, they are able to unite with the haptophore groups of circulating toxins. Consequently, they prevent the hapto-

phore groups of toxins from coming in contact with the cells, and thus intercept any poisonous effect the toxin might have. These cast-off cell receptors also act in the same manner outside of the body in which they originate, and that is why insensitiveness to toxins is transferable by means of serum (so-called passive immunization, applied in serum-therapy).

These antitoxins can be measured by means of a somewhat complicated method worked out by Ehrlich, the particulars of which are of minor interest to practitioners. Upon this method is based the titration of antitoxic therapeutic sera for Germany as it is applied in the Frankfurt Institute for Experimental Therapy.

These cast-off cell receptors, the antitoxins, which are supplied with only one haptophore group, are the simplest form of receptors. There are, however, others more complicated. In contradistinction to the latter, Ehrlich designates the antitoxins as receptors of the first order. Other cell receptors are cast oft after the injection of serum, bacilli, etc. Like the toxins, which may be considered cast-off receptors of bacilli, they possess, besides the haptophore group, a fermentative one with a special function (toxophore, agglutinating, precipitating, etc.). Such receptors are designated receptors of the second order.

Complicated Antibodies, Lysins, etc.—Still more complicated is the structure of the lysins, or receptors, cast off after the injection into the serum of cells of, for example, red blood-corpuscles, or even of bacteria. They are called receptors of the third order, and possess two haptophore groups, one of which adheres to the cell that is to be dissolved. The specific lytic action does not occur, however, until an activating ferment —the so-called complement—has been fixed to the second haptophore group.

In order that these various receptor apparatuses, with their different properties, might be united under one heading, the term 'antigen' has been introduced. By this term is understood a substance the introduction of which into the organism causes receptors of any order to be cast off. These cast-off receptors are frequently designated 'antibodies.' They may be receptors of the first order (antitoxins), or precipitins, agglutinins and lysins —*i.e.*, receptors of the second and third orders.

To avoid errors, it is therefore best to use the term 'reactive substances' (reagins) rather than 'antibodies,' since the latter implies a neutralization in the sense of an antitoxin.

Summary of Ehrlich's Lateral-Chain Theory.

The injection of toxin leads either to the death of the animal by poisoning or a casting off of cell receptors of the first order (antitoxins). The latter neutralize toxins in such a manner that a given amount of toxin and multiples thereof are saturated by a given amount of antitoxin and corresponding multiples (law of constant proportion). If the toxin has already reached the cell receptors, a separation of the toxin from the cell receptors is impossible or possible only by means of antitoxins in large quantities. The unsatisfactory results following the antitoxin-therapy in tetanus are due largely to this fact, since at the appearance of the first symptoms of tetanus the poison is already linked to the cell receptors.

After the injection of albuminous substances and bacteria, cell receptors of the second order, excepting lysins, are cast off. The receptors are precipitins and agglutinins, which have the property of producing a precipitation of albumen when brought together *in vitro* with the homologous albumen or bacteria or an agglutination of bacteria (see Chapter on Precipitins and Agglutinins).

The injection of body cells and bacteria causes the casting off, not only of receptors of the second order (agglutinins and precipitins), but also of receptors of the third order (lysins), which produce a lysis of the elements after complement has been added—*e.g.*, under the *z*ytolysin group, hæmolysins after an injection of erythrocytes; also bacteriolysins, etc. Or, if lysis does not occur, the approach of receptors of the third order to the elements in question prepares them for phagocytosis (see also Nicolle's theory in Chapter IV., on Hypersensitiveness).

It is not inconsistent with Ehrlich's lateral-chain theory to assume that only such cells as are sensitive to toxic effects can produce antitoxins or antibodies. This view met with a great deal of opposition, particularly from Buchner and Gruber, who insisted upon antitoxin production for the very cells not influenced by toxin. As more recent investigations have shown, those cells which have not been especially

altered by the toxic effect actually seem to participate very materially in the production of antibodies.

Further objections were raised to the assumption of the preformation of the various receptors, which, according to Ehrlich, would serve in ordinary life as receptors for the ingestion of foodstuffs. The assumption does indeed seem somewhat improbable, since the ingestion of food does not take place through the receptors, and we know that this form of reabsorption of albumen arises only when the normal mechanism of intestinal reabsorption is disordered (see Chapter VI., on Precipitins). Gruber and others assume that receptors are formed only under toxic stimulation, yet Ehrlich's assumption explains these processes more satisfactorily.

Receptors and the Ingestion of Food.—The relation of the action of receptors to the ingestion of food is made obvious by the historical genesis of the lateral-chain theory. Influenced by organic chemistry, particularly colour chemistry, Ehrlich assumed that there were a chief nucleus and side-chains in protoplasm. In the chief nucleus no alterations can occur without infringing upon the lives of the cells themselves. Oxidation and reduction take place in the lateral chains of the protoplasm, as Ehrlich showed by experiments in colour analysis in 1882. This process, Ehrlich assumed, was an indication of cell nutrition. However, in the light of further development of the lateral-chain theory, it appears more probable that cellular nutrition does not take place through the receptors, but by diffusion and dialysis.

SURVEY OF THE VARIOUS RECEPTOR APPARATUS.

Injection of a substance of antigenic character.

Receptors.			
First order	... Antitoxins	...	After injection of toxin.
Second order	... Precipitins	...	After injection of albumen.
	Agglutinins	...	After injection of bacteria.
Third order	... Lysins*	...	After injection of cells, Cytolysins: Hæmolysins, Spermolysins, Nephrolysins, Hepatolysins, etc.
			After injection of bacteria, Bacteriolysins: Typholysin, Choleralysin, etc.

Called also: Intermediate bodies, Immune bodies, Amboceptors, Preparator, Fixateur, Desmon, Substance sensibilatrice.

* These act specifically only when the activating ferment—the complement, also called cytase and alexin—is added.

The table (p. 35) presents a survey of the various reacting substances originating after the incorporation of different materials. It also gives one an idea of the large number of terms applied to these substances, making the subject of immunizing processes extremely complicated for anyone who does not understand the synonymity of the terms. (*Cf.* quotation from Deycke and Much in Preface.)

It is also observed that the injection of a lysin produces an antilysin. In a similar manner, the injection of an amboceptor is followed by the production of an anti-amboceptor. However, on account of experimental difficulties, these phenomena have never been satisfactorily explained.

CHAPTER IV

Hypersensitiveness: Definition — Various forms — Vasomotor nature of diseases dependent upon hypersensitiveness and the question of specificity—Proofs of the vasomotor nature of phenomena of hypersensitiveness—Theories of nature and origin—Attempts to prevent occurrence of hypersensitiveness—Its practical significance—Passive transmission—Incubation.

HYPERSENSITIVENESS is a natural phenomenon, the significance of which in immunity research and the subject of immunity in general cannot be overestimated. It is only in the past few years that the attention of investigators has been directed to these phenomena and their laws. The historical development of the subject of hypersensitiveness is discussed elsewhere (p. 83).

Definition of Hypersensitiveness.

By *hypersensitiveness* is meant the condition of an organism upon which an excitant has acted one or more times, the organism reacting more strongly at each repetition of the excitant than does another organism of the same kind upon which the same excitant is acting for the *first* time. To merit the title 'hypersensitiveness,' the difference in reaction must be so obvious that it cannot be ascribed to individual differences of hypersensitiveness. The conditions arising after the action of a stimulus are as follows: The body as a rule reacts by adapting itself to the stimuli most frequently affecting it (adaptation, accommodation, or acclimatization). These adaptations to excitants generally concern physical conditions, such as heat, cold, wind, dust, etc. The same law holds good, however, for numerous chemical agents as well.

Every physician has to reckon daily with this well-known fact, as, for example, in case he wishes to use any remedy for a long time. The mechanism of these adaptations is varied. So far as adaptation to chemical poisons—*e.g.*, to alcohol or morphine—is concerned, the cells become accustomed to living with a certain concentration of the substance circulating in the juices, as can be easily proved by experiments upon unicellular organisms. In a properly arranged experiment they live undisturbed in a concentration of a substance which, with insufficient adaptation, would act as a poison. It follows from this that tolerance must imply an adaptation to the ingestion of this poison, since, according to a well-known biologic law, any alteration of the concentration of juices isotonic for the cells acts as a stimulus, which calls for additional quantities of the substance.

Adaptation to Toxins.—An entirely different, but very important form of adaptation takes place toward bacterial poisons (toxins : see Chapter III., on Lateral-Chain Theory). We have to deal here with high molecular poisons secreted by bacteria, which cannot be diffused in the cells like salt solutions. They are fixed by receptors which adhere to the cells, and in this manner the toxic effect is exerted upon the cells. Under certain conditions these cell receptors are cast off into the serum as free receptors. The fixation of the poison then occurs at a distance from the cell, and there is no toxic action upon the cell. In these few words is contained the kernel of Ehrlich's lateral-chain theory—that ingenious figure portraying conditions found in experimental work—and an explanation offered for the fact that in insensitiveness to toxins (in so-called toxic immunity) a toxæmia (avidity for toxins) occurs, which is not analogous to morphinism. In tolerance to toxins the cells are not accustomed to another concentration of juices, and therefore have no hunger for toxins; on the contrary, the cell manifests its hunger for salts—whether common salt, morphine, or other salts—when alteration occurs in the concentration, even if this alteration consists in a return to normal conditions.

A hypersensitiveness occurs after the injection of a heterogeneous albuminous substance (serum, albumen of an organ,

or bacterial albumen), and becomes manifest on repetition of the injection.

This concise definition of hypersensitiveness, given by the author, was in direct opposition to the view held up to that time—that the injection of an antigen led to hypersensitiveness.

Naturally this definition is too brief to include all the details. The results following the injection of a toxin are not fully enumerated. It might, perhaps, be more exact to say the injection of a heterogeneous albuminous substance eliciting the formation of lytic antibodies leads to hypersensitiveness, but this definition would include my theory as to the character of hypersensitiveness, which has yet to be proved correct.

The manifestations of hypersensitiveness are further modified very essentially in proportion to the susceptibility of the individual to the toxic action of the substance liberated, after repeated injections, by sessile receptors, etc.

Investigators were slow to understand the phenomena of hypersensitiveness for the reason that in this condition complicated cellular phenomena have to be dealt with, a study of which is difficult for the reason that we are unable to observe and explain the processes taking place in the individual cell, but are restricted to conclusions drawn from reactions in the processes occurring in the cells. We have now reached the point where we have enough material to give us a well-defined and outlined idea of hypersensitiveness, but no agreement has been reached as to its true character, although we have much valuable data on the subject.

Various Forms of Hypersensitiveness.

We are now familiar with various forms of hypersensitiveness. We may say, briefly, that hypersensitiveness may manifest itself in two forms, of quite different significance to the individual. The symptoms may be associated either with a rapid course of the reactive phenomena, or, more important still, the phenomena may *run a more stormy course.*

In the latter case the reaction of hypersensitiveness may

become so intense that the death of the organism follows. All the heterogeneous albuminous substances (sero-albumen, organ albumen, and bacterial albumen) have the property of producing hypersensitiveness, which becomes manifest after one or more injections (reinjection). The symptom-complex of hypersensitiveness was described by Wolff-Eisner in 1904 as follows:

'**Severe Form of Hypersensitiveness.**—After a short incubation stage, often lasting but a few minutes, a severe dyspnœa follows abruptly, similar to that following air-embolus. Death sometimes occurs after a few moments, with tonic-clonic convulsions. In other cases the dyspnœa is transitory: the rabbits often recover in a surprisingly short time—only a few hours—and appear perfectly normal. If, however, the next injection is made some time after, the same symptoms occur, with still more intensity, and the animal succumbs' (*Centr. f. Bakt.*, vol. xxxvii., Nos. 3, 4 and 5).

This brief description is so inclusive that even now, after very elaborate work done in this line, nothing needs to be added; and Kraus has also quoted this paragraph, not only in justice to the historical fact, but to describe the phenomena of hypersensitiveness in the most concise manner possible.

There are *no essential differences* between that hypersensitiveness characterized by the time of the reaction and the form running a fatal course. Whatever form of hypersensitiveness becomes manifest is entirely dependent upon the toxicity of the albumen applied, and Wolff-Eisner in 1904 emphasized the fact that sero-albumen is the least toxic,* the body substance of certain bacteria and sperma most toxic. The reaction depends also upon the quantity applied in the re-injection and upon the individual susceptibility of the organism, which will be discussed more fully later on. Both forms of hypersensitiveness were studied at the same time, in 1904—viz., the form of hypersensitiveness occurring after the injection of larger amounts of albumen into an animal, terminating fatally (see above); and the form described by Pirquet, the serum disease produced by sero-

* *I.e.*, elicits slower and less intensive phenomena.

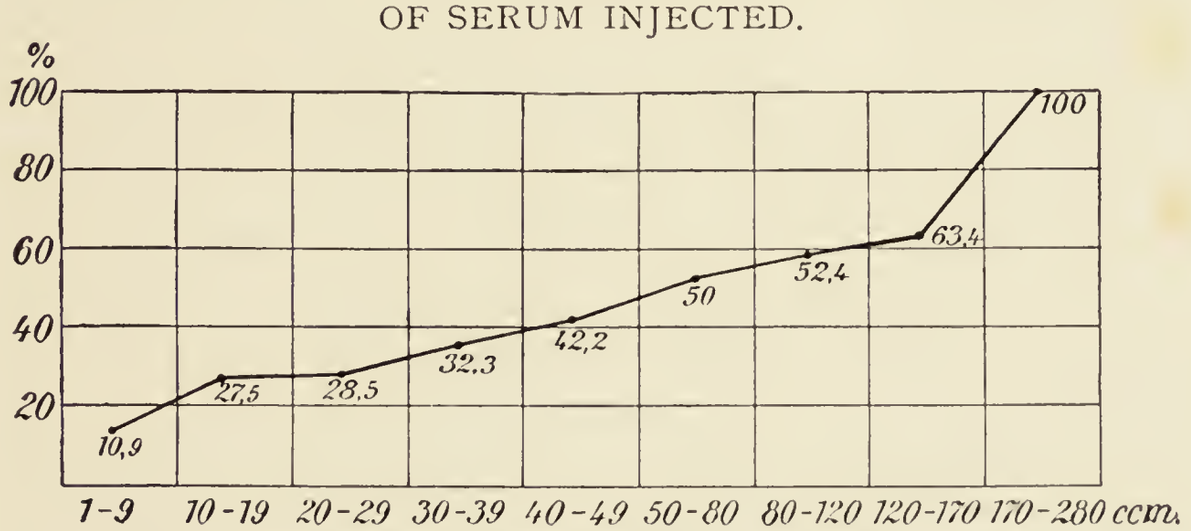
albumen only slightly toxic (see above; Pirquet and Schick, 'Serumkrankheit,' Vienna, 1906; 'Studien über Vaccination,' Vienna, 1907). Owing to special conditions, Pirquet made it possible to carry out observations on human beings.

Serum Disease.—Although serum disease had been known for a long time, the credit is due to Pirquet for having attributed the phenomena of the disease and its various clinical manifestations to an increased and accelerated reaction—that is to say, ascribing them to the phenomena of hypersensitiveness.

Vasomotor Nature of Diseases of Hypersensitiveness and the Question of Specificity.

Serum disease is the type of a disease of hypersensitiveness experimentally produced. It runs a course with fever, exanthema, swelling of the glands, and irritation of the kidneys, thus differing very little in its clinical picture from an infectious disease.

DEPENDENCE OF SERUM DISEASE UPON THE QUANTITY OF SERUM INJECTED.



Amount of serum used for first injection.

Six hundred and ninety-two cases were used for the curve made from material supplied by Weaver (*Arch. of Internat. Med.*, vol. iii., No. 5).

Relation of Quantity Injected to Serum Disease.—The symptoms of serum disease do not appear uniformly in all individuals after serum injection; moreover, a predisposition is necessary. However, we are now in a position to come nearer a solution of the problem which predisposition has always been to the clinician. It has been ascertained

that the more intense the symptoms shown by human beings at the first injection, the larger the dose has been. There is no doubt that Pirquet performed the greatest test ever made in the field of serum disease, when at Escherich's clinic, in the application of Moser's scarlatina serum, enormous quantities of heterogeneous serum were injected. The curve given on p. 41 shows very distinctly the conditions referred to.

If symptoms of serum disease do not appear after the initial injection in small doses, they occur in increasing number after repeated injections. Although they may be forced by high initial doses, they occur *in practically* 100 *per cent. of cases where smaller doses are used after the fourth or fifth injection*, provided, of course, that the injections do not immediately follow each other, but succeed at intervals of not less than eight or nine days, corresponding to the period of incubation in serum disease, during which the antibodies are formed, without which serum disease cannot occur. There is, of course, no absolute insensitiveness to a heterogeneous serum that may not be overcome by increasing the dose; much less is insensitiveness acquired. On the contrary, there arises a hypersensitiveness, as after the injection of any heterogeneous albumen, characterized by increased or accelerated reaction symptoms, *or by the occurrence of the same reactions after either small or large doses.*

Serum disease is not always unattended by danger, as scholastic medicine dogmatically proclaimed, unopposed even by Pirquet, who conceded a certain danger in intravenous injection only.

After the initial injection, to be sure, severe general symptoms are rare; yet there have been cases where the patient died in from ten to fifteen minutes after the serum injection.

Dangers of Serum Disease in Serum-Therapy.—The symptoms consist of redness of the skin, particularly of the face, dyspnœa, urticaria, and œdema, especially of the face. The mucosa of the pharynx and larynx becomes swollen. Death ensues through paralysis of the respiratory organs, the heart continuing to beat for some time.

The symptoms are exactly analogous to those occurring in the rabbit experimentally made hypersensitive (see above).

Rosenau and Anderson (Bull. 29, Hyg. Lab., Washington) reported in 1906 nineteen cases of this kind from literature. Gillette (*Ther. Gaz.*, 1909, vol. xxxiii., p. 159) reported twenty-eight cases with severe general reaction, sixteen of which were fatal. The author has reported an analogous case in the 'Manual of Serum-Therapy,' München, 1910 (see also Besche, 'Gefahrdrohende Dyspnoe und Collaps nach Serumerstinjektion,' *Berlin. Klin. Woch.*, 1909, No. 36).

After the initial injection these severe phenomena seldom occur. Nevertheless, Weaver's proposition is worthy of note—viz., that a small amount of serum be first injected in order to make sure there will be no such speedy general reaction. It is safe to say that such symptoms necessarily occur more frequently in reinjections, and cases will soon be met with in German literature, which has always attributed these serum symptoms to the disease for which the serum was applied. Warnings have been sounded in American literature against applying serum injections in asthma. This empirical dictum, however, says nothing as to the underlying reasons. It seems to me that caution should be observed more especially in neuropathic predisposition, particularly with unstable vasomotor systems (for details see 'Specificity of Hypersensitiveness,' p. 46).

It is easy to satisfy oneself of the presence of a hypersensitiveness to horse serum by injecting intracutaneously $\frac{1}{10}$ c.c. of the serum. While reaction occurs in sensitive persons at the site of injection in from five to seven days, in hypersensitive persons it is hastened or increased (Wolff-Eisner). It is advisable in suitable cases to apply Wolff-Eisner's method (from experiments not yet published).*

Diseases of Hypersensitiveness to Albumen.—There is, however, a long list of *spontaneously occurring diseases of hyper-*

* *Cf.* Rosenau, 'Further Studies upon Anaphylaxis,' Bull. No. 45, Hyg. Lab., Washington, 1908. Rosenau succeeded in producing in guinea-pigs symptoms of hypersensitiveness somewhat analogous to those of eclampsia by injections of their own placentar substance.

sensitiveness of clinical importance which are not induced by experimental injections of albuminous substances. Wolff-Eisner (J. F. Lehmann, 1906) was the first to offer convincing proof that in the affection of hay-fever we had to deal with a condition of hypersensitiveness to albumen, caused by the repeated absorption of pollen albumen. The same author also pointed out that the absorption of heterogeneous albumen plays a part in the etiology of urticaria (*Dermat. Centr.*, vol x., No. 6), and that from this point of view all the numerous factors of urticaria could be classified in one group. Recent developments have shown how well this opinion was justified—*i.e.*, that hypersensitiveness to albumen was of great *clinical* significance. It is very probable that those authors (Liepmann and others) who attributed eclampsia to the action of a specific poison present only in the eclamptic placenta were wrong, and that Rosenau was right in referring the symptoms of eclampsia to a repeated reabsorption of villi—a view which harmonizes perfectly with the anatomico-pathologic conditions (Schmorl and Weichardt).*

Salt-Fever in Infants.—Finkelstein recently described an interesting syndrome in infants—a fever following the ingestion of salt and sugar—and Friedemann (*Arch. f. Hyg.*, 1909) has proved that these symptoms occur more especially in animals that have been previously treated with albumen, and are thus made hypersensitive. From these investigations it would seem probable that Finkelstein's cases had to do with children affected with intestinal disorders, causing heterogeneous albumen to be reabsorbed, in consequence of which the heat centres were unstable.

Anaphylactic Fall of Temperature.—Observation of the temperature curve plays a part in the detection of the finest symptoms of hypersensitiveness. Pfeiffer and Finsterer, in works which will be cited later, called attention to the significance of temperature in determining hypersensitiveness, viz., the so-called anaphylactic temperature shock. Here, too, hypersensitiveness is characterized by a manifestation of the symptoms from relatively small sensibilizing doses, while no specificity

* This explanation is preferable to that of Klemperer and Otto, because of its simplicity and safety.

exists, since, as has already been mentioned, larger doses of heterogeneous albuminous substances, so far as they are reabsorbable, tend to lower the temperature. This fully explains Ranzi's objections (*Wien. Klin. Woch.*, 1909, No. 40).

Pfeiffer's observation is, therefore, only one particular instance of the law governing hypersensitiveness—viz., that in reinjections small doses act as very large ones otherwise would. Pfeiffer's law is not confirmed in the use of tuberculin in human beings, for in such cases, in spite of previous sensibilization, hypersensitiveness is manifested by a rise of temperature rather than a decrease. Pfeiffer's statement is an interesting and important detail, but not a fundamental law.

Vasomotor System and Hypersensitiveness.—Hypersensitiveness offers a rich field for further investigations, when one considers the influence which the reabsorption of heterogeneous albuminous substances exerts upon the vasomotor system. The fact that after the ingestion of heterogeneous substances such a predisposition arises that fever appears after the ingestion of salt or sugar, suggests that an instability of the vasomotor centres may be produced in this way. Until very recently nothing was known regarding this vasomotor instability. Now we can at least say that individual differences of hypersensitiveness may be explained by differences in vasomotor sensitiveness, and *that individuals with an unstable vasomotor system are especially predisposed to the more severe forms of hypersensitiveness.*

This is really explained by the fact that the pathological alterations observed in fatal cases of hypersensitiveness, particularly the capillary changes in the lungs found by the author, leading to an obliteration of the alveolar lumen, and resembling macroscopically pneumonic hepatization, can be ascribed only to vasomotor influence. The vasomotor nature of hypersensitiveness is also attested by the fact that the threatening hypersensitive symptoms following a reinjection completely subside in a few minutes, provided death does not ensue. Wolff-Eisner in 1904 ascribed all symptoms of hypersensitiveness and *all* effects due to *heterogeneous albumen, including bacteria,* to central causes. The maximum vasomotor dilatation occurs centrally, as does the fall of tempera-

ture, to which Pfeiffer recently attracted attention (*Wien. Klin. Woch.*, 1909, No. 40). The fall of temperature, however, should not be considered characteristic of a reaction. It accompanies a hypersensitive shock (see above), while doses with less effect may even produce a rise of temperature.*

The phenomena of hypersensitiveness were ascribed by Wolff-Eisner to the vasomotor centre; Passler attributed the cardiac symptoms, as found in diphtheria, to vasomotor irritation or paralysis.

The clinical significance of hypersensitiveness does not terminate here. We may indicate very briefly the rôle which hypersensitiveness plays in tuberculin diagnosis, and more particularly in the course of tuberculosis.

The Specificity of Hypersensitiveness.

There is a specificity of hypersensitiveness in much the same sense as there is a specificity in the phenomena of agglutination—*i.e.*, we cannot produce a typical hypersensitiveness to the body substance of typhoid bacilli by means of sero-albumen. On the other hand, there are here, as in agglutination, certain group reactions if the injections are frequently repeated. Whether these phenomena are explained by the affinity of various albuminous substances, as is the case in precipitation, or by a vasomotor instability due to the introduction of serum, is yet to be determined.†

Many facts have recently been reported which in themselves contradict the specificity of hypersensitiveness, and have, to a certain extent, been used as arguments against it.

According to Kraus (*Zeits. f. Immun. Forsch.*, 1909, vol. iii., No. 2), animals previously treated with heterogeneous serum and bouillon show, twenty-four hours after injections of typhoid and

* Braun's claim that the credit for ascribing hypersensitiveness to the brain is due to Besredka is therefore incorrect. His publications in the *Annales de l'Institut Pasteur* did not appear until 1907-08.

† The phenomenon of Theobald Smith—serum hypersensitiveness occurring together with an injection of toxin—is only an incident in hypersensitiveness, and can only be mentioned casually in a survey of this subject. It appears that the formation of reactive bodies is quantitatively increased by the injection of toxins (Otto and Braun, *Münch. Med. Woch.*, 1909, No. 37).

cholera toxin and tuberculin, symptoms very similar to those of anaphylaxis. Kraus attributes this to an increase of sensitiveness to poison, caused by heterogeneous serum. Similar results have been reported by Arthus and Delanöe (*Acad. des Scien.*, Paris, 1909).

Kraus's assumption explains nothing. However, the facts reported tend to support our opinion that the symptoms of hypersensitiveness are attributable to vasomotor influences. The practical significance of this theory, and the facts upon which the view is based, may be given here.

Evidence in Favour of the Vasomotor Basis of Hypersensitiveness.

1. Clinical Experimental Evidence.

As has already been stated, the most severe phenomena of hypersensitiveness occur after repeated injections of heterogeneous albumen. The symptoms may pass off of themselves, with the aid of artificial respiration, and be followed by perfect health. The heart continues to functionate quietly. This is the only explanation of the resuscitation of man or animal by means of artificial respiration in severe cases of hypersensitiveness.

Although the animal may entirely recover from a hypersensitive shock and appear to be in perfect health, there is still an increased sensitiveness to injections of any heterogeneous albumen (see above : Kraus, Arthus, and Delanöe). We have stated that any insensitiveness after the initial injection is only apparent, and may be entirely overcome by larger doses. We may conclude from this that the centres become more sensitive because of the anaphylactic shock, so that they react to smaller doses. It may also be mentioned that animals previously treated with heterogeneous albumen—*e.g.*, tuberculin—and exhibiting no morbid symptoms become so sensitive to concussions that they die instantly from a fall of a few feet—one which animals not so treated would bear without any serious consequences (Wolff-Eisner : unpublished experiments).

2. Clinical Evidence.

The preceding arguments are merely evidence that the symptoms of hypersensitiveness originate centrally. Further proofs are derived from the fact that the vasomotor centres are especially concerned in the production of hypersensitiveness.

The clinical observations furnish important evidence in favour of these assumptions. We note in the first place that individuals with an unstable vasomotor system—*i.e.*, persons who turn pale or red very easily, with evanescent flushing and increased reflex irritability in general, and of the vasomotor system in particular, or individuals who show dermography, swelling of the nasal turbinates, etc.—are especially predisposed to serum disease, and that the same individuals, especially in infancy, are very sensitive to visceral ingestion of albumen, and exhibit urticaria with the slightest intestinal disorders. Cow's milk, for instance, does not agree with such children, but only that of mother or nurse. (*Cf.* Chapter VI., on Precipitins.)

Clinicians have known for a long while that there is a relation between asthma nervosum, urticaria, fibrinous bronchitis, and membranous enteritis. This relationship is an intimate one, since eosinophile cells are found in abundance in asthma sputum and in the membranes in membranous enteritis. The nature of this relationship was formerly very puzzling, for the 'neurotic factor' probably satisfied no one who thoroughly investigated the matter.

These diseases, however different their symptoms, are due wholly to a vasomotor irritability and to vasomotor disturbances, which are responsible for the eosinophile secretions and the fibrinous exudate, and the spastic condition as well. On the other hand, in these conditions, particularly in asthma, sensitiveness to the injection of heterogeneous albumen is so great that, according to the reports on the subject, an injection must be actually regarded as dangerous. It may be mentioned in this connection that the severe symptoms following the resorption of pollen likewise occur

only in nervous (vasomotor) individuals. It is no mere coincidence that asthma may, and indeed does, occur in most moderate and severe cases of pollen sensitiveness.

A further series of important proofs can be derived from a study of the clinical symptoms in consumptives and of the tuberculin reaction. It is well known that the tubercular subject is an example of vasomotor irritability. He shows all the typical symptoms, such as an unstable condition of the vasomotor system, evanescent erythema, irregularities of temperature, etc. Very likely this irritability of the vasomotor centre is caused by the toxins of the tubercle bacillus (the tuberculin, which is an endotoxin).

In this case also the sensitiveness to all other heterogeneous albuminous substances is increased by the influence of tuberculin—*i.e.*, the consumptive reacts more readily to such irritations than does a healthy individual. For example, he is more sensitive to injections of peptone than a normal person. Because of the fact that tubercular persons show great variations of temperature after injections of peptone, and react to smaller quantities than do normal individuals, tuberculin action was wrongly regarded as peptone reaction.

3. Pathologic-Anatomical Evidence.

According to the author's own statements, a maximum dilatation of the lung capillaries is found after death from hypersensitiveness. This he regarded as characteristic of death from hypersensitiveness, but did not make a more detailed publication, having been informed by L. Pick that the same thing was to be observed in cases of death from hanging. Now, however, the case seems sufficiently clear. Vasodilatation is not in itself characteristic of death from hypersensitiveness, but only of the vasomotor paralysis. It is irrelevant, so far as the anatomical findings are concerned, whether the influence upon the vasomotor centre is due to hypersensitiveness or to the irritation of the centre by carbon dioxide.

Theories of the Origin and Character of Hypersensitiveness.

To those authors who gave us our first knowledge of hypersensitiveness we owe the first theories of its nature. Pirquet ascribed hypersensitiveness, particularly serum disease, to the union of antibody and antigen.

Wolff-Eisner departed from the analogies existing between the injection of morphologically organized albumen and albumen not morphotically organized. In morphologically organized albumen, such as red blood-corpuscles or typhoid bacilli, lysis is demonstrable after repeated injections, a lysis occurring together with the other clinical signs of hypersensitiveness. Since the same clinical symptoms occur in the example just cited (typhoid bacilli and red blood-corpuscles), the author regards serum albumen as only apparently dissolved, and assumes that it is only reabsorbable through the agency of a lysin (so-called albumino-lysins). This conception solves the problem of a great many phenomena which cannot otherwise be explained. What is more, Pirquet's somewhat confusing conception is avoided—viz., that the union of antibody and antigen may produce a reaction.

The author's most recent theory is as follows: Two factors are concerned—viz., (1) lysis. This is an indispensable requirement for the production of the phenomena of hypersensitiveness. (2) The reaction upon substances made reabsorbable by lysis.

The manner in which the reabsorbable albumen acts upon the organism is dependent upon individual factors, and is of a vital nature, as yet not well-defined. Therefore, hypersensitiveness is not a direct effect of the lysin content. The same view is held by Much (see 'Immunity,' p. 148).

French authors, in explaining hypersensitiveness, often speak of an 'anaphylactic reactive body' or 'anaphylactin,' but offer no information of any value in regard to the nature of the phenomenon. The same is true of Pirquet's term 'allergie.' The name has been widely adopted, to be sure, but it has nothing to do with the nature of hypersensitive-

ness. It is merely a foreign word meaning 'hypersensitiveness,' and the many who suppose that the nature of hypersensitiveness is explained by the term are misled.*

Nicolle's theory should also be mentioned. This theory is based upon a lytic nature of hypersensitiveness, and attributes the origin of hypersensitiveness or immunity directly to the predominance of lysin or agglutinin formation after the injection of a substance. According to Wolff-Eisner's view, lysins produce hypersensitiveness; agglutinins, insensitiveness.

Attempts to Prevent the Occurrence of Hypersensitiveness.

Owing to the clinical importance of hypersensitiveness, many attempts have been made to prevent the occurrence of this condition. For example, experiments have been instituted to isolate from albumen certain substances which were supposed to give rise to hypersensitiveness (Besredka). These experiments have thus far miscarried, and it seems there is little chance of their success. However, experiments for preventing hypersensitiveness by the injection of small doses have been more successful. The severe symptoms of hypersensitiveness resulting in death are apparently caused by central influences, and for this reason Wolff-Eisner's findings are of importance—viz., that the heterogeneous albumen may, under certain conditions, be fixed to local receptors, thus preventing its action centrally.

* Pirquet's and Wolff-Eisner's views coincide in that they do not, as do French authors, suppose a special toxin to produce hypersensitiveness (Richet's aptotoxin), but attribute hypersensitiveness to a union of antigen (albumen) and antibody (reactive substance in the serum of the individual injected). (Pirquet made the claim for serum disease, revaccination, and cutaneous tuberculin reaction; Wolff-Eisner for hay-fever, tuberculin reactions, and the clinical phenomena of tuberculosis.) The differences in the views of the two investigators are that Pirquet regards the process as somewhat analogous to the union *in vitro* of precipitable substances and precipitin (see 'Serumkrankheit,' p. 113), without identifying the vital antibodies with precipitins, while Wolff-Eisner does not consider the process analogous to precipitation, but to bacteriolysis. Although these views are somewhat similar, their differences are of great significance. In his theoretical remarks upon revaccination, Pirquet has taken almost the same view-point as Wolff-Eisner.

Besredka's findings can be explained by the central action. According to these findings, death from hypersensitiveness can be prevented by narcosis. It is apparently possible to stupefy the central nerve cells by narcotics, and thus to carry the imperilled cells past the critical moment.* Besredka has maintained that, under certain circumstances, it is possible to prevent the occurrence of hypersensitiveness by the injection of chemicals. Other authors have not confirmed this (Rosenau).

The Practical Significance of Hypersensitiveness.

The great practical significance of hypersensitiveness is obvious from the symptom-complex above described. Now that we have an exact knowledge of the manifestations of hypersensitiveness, the number of diseases which must be ascribed to this condition is steadily increasing. We must also take hypersensitiveness into account in applying serum-therapy. Owing to the present technique of preparing sera, all the commercial products have been obtained from horses. Persons who have been injected repeatedly with horse serum frequently exhibit hypersensitive symptoms. This seemed unavoidable, and there have been cases of serum disease that were of anything but a harmless character. Serum disease may occur with severity in persons with a great vasomotor instability (see above).

The Object of Hypersensitiveness.—According to what has been said with regard to the diseases due to hypersensitiveness, the idea might arise that the phenomenon is abnormal and extremely dangerous to the existence of the individual affected. As we have seen, this is true in many cases. Nevertheless, this view is not altogether justified, for we must bear in mind that, apart from a few cases, and except for pathologic conditions, Nature has taken care that heterogeneous albuminous substances should not enter the body in too large quantities. Apart from pollen disease,

* *Cf.* narcosis in eclampsia. According to Rosenau, however, narcosis is not a sure preventive of death in hypersensitiveness any more than in eclampsia.

it enters the body practically only in cases of snake-bites and stings of insects, and a system of regulation in the intestinal tract prevents the reabsorption of heterogeneous albumen insufficiently broken up. Consequently, bacteria represent the only remaining factor for bringing heterogeneous albumen into reactive contact with the animal organism, and hypersensitiveness serves a very valuable purpose in this respect. If, for example, a bacillus seeks to enter the body, and multiplies only to a slight extent, hypersensitiveness manifests itself at the site of colonization by a rapid and intense inflammation and a liberation of the protective agents of the body, and their concentration at the point of attack follows. So high a degree of protection of the body against infections could be attained in no other way. Under certain conditions, hypersensitiveness might certainly be of disadvantage to the body, as, for instance, in case bacilli should invade the body in great numbers. This does not, however, alter the fact that in the vast majority of cases hypersensitiveness acts as an extremely efficient protective agent. This view is beginning to be accepted by expert investigators, notable among whom is Römer, who has called attention to the consequence of these views in tuberculosis infection and immunity.

Passive Transmission of Hypersensitiveness.

Some interesting tests made recently seem to have proved that passive transmission of hypersensitiveness is possible. From this we are justified in assuming that hypersensitiveness may be ascribed to a reacting substance present in serum.

The reports include passive transmission of hypersensitiveness in eclampsia (Weichardt), in tuberculosis (Yamamouchi, and Bauer, *Münch. Med. Woch.*, 1909, No. 24), in cancer (Pfeiffer and Finsterer, *Wien. Klin. Woch.*, 1909, Nos. 28, 36, and 40), and in crepitin (Richet, *Soc. de Biol.*, 1909).

Richet's contention of having been the first to report the passive transmission of hypersensitiveness has therefore no objective basis.

Reports in literature and numerous experiments of my own prove, however, that we cannot count on a constancy of this transmission. Not only must reacting substances (reagins) be present, but there must also be a special sensitiveness of the central nervous system to the substances liberated by the action of the reagents (see above). Here, too, it becomes apparent that two factors must always co-operate in the origin of hypersensitiveness, as the author has postulated (see Early Diagnosis and Tuberculosis Immunity).

It is becoming more and more obvious that these reacting substances are to be regarded as lysins in the sense first suggested by the author. Except for the reasons mentioned for serum hypersensitiveness and the action of tuberculin, his statement confirms the fact that the pressed juice of the brain* acts more intensely and rapidly than the Latapie grinding.† Brown has recently reported a fact which may be regarded as agreeing with the theories of Wolff-Eisner and Nicolle. Serum disease occurs very readily in organisms in which the precipitin formation is less intensive—*e.g.*, human beings or guinea-pigs; it is more difficult to produce in rabbits, which form coagulins very easily. The significance of lysins in the origin of hypersensitiveness is evidenced in the experiments reported, yet the contrast in the action of lysins and coagulins, as described by Nicolle, is at the same time made apparent.

Incubation.

The symptoms of serum disease do not appear immediately after the injection, but there follows a so-called period of incubation. First of all, local symptoms often become manifest, the general phenomena appearing after an incubation period of from six to twelve days.

By incubation was generally implied that period of time elapsing after infection during which a proliferation of the

* Made with the Buchner press.

† A mechanical fragmentation of an organ, made with a grinding machine (*broyeur latapie*).

exciters of infection took place; and only after this proliferation had reached a certain degree was the disease made apparent by the manifestation of symptoms. For a long time this incubation theory was generally accepted, and the production of disease ascribed to the proliferation of living, unchanged infection exciters. In serum disease, however, absolutely no proliferating substance exists, yet there is a period of incubation. The author has long believed that the symptoms of an infectious disease become apparent when the body juices become intimately associated with the invading substances, so that a contact reaction takes place, as, for instance, when the body has formed bacteriolysins and a bacteriolysis has begun. In serum disease particularly Pirquet also takes the ground that the symptoms appear when the antibody production takes place.

An incubation period also follows the injection of toxins. This has been explained by the fact that a number of organs are capable of binding the toxins, but that this fixation is partially reversible and transitory, therefore chemical rather than physical, and that the general symptoms occur only after the poison has reached the nerve centres.

Fixations of this sort to endotoxins and albuminous substances of a non-toxic nature are also found. Hence the phenomenon of incubation is composed of two elements, upon which the duration of the incubation depends: (1) The presence of bacteriolysins, to which the liberation of endotoxins is due; and (2) the entrance of endotoxins into central points after passing the barriers presented by any sessile receptors that may have been present.

Incubation in Reinjection.—After reinjection (repeated injection) the incubation period is shortened. The symptoms of the initial disease reappear earlier, sometimes immediately, and may be more transitory. They may occur in the form of local or general reactions, according to Pirquet. Reinjections made before the symptoms of serum disease become manifest—*i.e.*, during the period of incubation—exert no action whatever. Many erroneous beliefs as to the harmlessness or inefficacy of serum reinjections, and in regard to the

repeated injections of heterogeneous albumen, tuberculin, etc., depend upon this fact.

After this survey of the fundamental principles of hypersensitiveness, we shall cite a few examples by means of which the laws of hypersensitiveness have been converted from clinical enigmas to readily understood symptom-complexes.

CHAPTER V

Clinical symptom-complexes dependent upon hypersensitiveness.

Hay-Fever.

In pollen disease we have to deal with a typical hypersensitiveness to albumen. This disease is significant in that it was the first affection in which a hypersensitiveness of this character was proved to exist. The sensitiveness to pollen substance is heightened in the course of a single attack, as well as in the course of the disease, so that, as a rule, in time an increase of the phenomena occurs.

Etiology.—There have been few diseases with as many theories of etiology. Blackley was the first to point out that the pollen of grasses and corn was especially responsible for hay-fever. The investigations of Dunbar, Weichardt, Wolff-Eisner, and others, have confirmed the etiological significance of pollen, and at the present time the etiology of the disease is generally understood. In place of the misleading term 'hay-fever,' the name of the affection should be 'pollen disease.' It is by no means a rare disease, being extremely common, not only in England and America, but in Germany and other countries. The disease consists of a conjunctivitis, rhinitis, and asthmatic conditions, caused by the action of the pollen. The peculiarity of the affection, however, does not consist in the rhinitis and conjunctivitis, but, as in the conjunctival reaction to tuberculin, in the production of this inflammatory reaction by means of pollen. The diagnosis is made by testing the patient's sensitiveness to pollen substance.

As we know, the majority of people are not affected by pollen. This, however, is not because substances antitoxic

to pollen circulate in the blood, but because there is present an insensitiveness to the poison, described by Wolff-Eisner as similar to the lack of sensitiveness to tuberculin of man and animals in perfect health (absence of lytic—*i.e.*, liberating —amboceptors).

Rôle of Predisposition.—Individuals with an unstable vasomotor system are especially predisposed to pollen disease. A vasomotor coryza cannot be distinguished from hay-fever without the pollen-test. The etiological therapy consists in the elimination of pollen by protective apparatus or climatic therapy.

Serum-Therapy in Hay-Fever.—We may mention some of the attempts made to control hay-fever by serum-therapy. Two sera are employed for this purpose. Their subcutaneous injection affords no protection whatever against pollen disease, nor has it any curative effect upon the disease. To obtain any result the serum must be applied locally.

Dunbar's pollantin is obtained by treating horses with increasing doses of pollen toxin.

Weichardt's graminol is the serum of ruminants liberated from salts by dialysis and dried in a vacuum.

Both are sedatives; both are without effect on many individuals; both act as irritants in many cases, pollantin more frequently than graminol.*

Mode of Action of Hay-Fever Sera.—Pollantin is not an antitoxic serum. This follows from the frequent failure of the serum to relieve pollen disease, even when used as a prophylactic. This in itself precludes the possibility of its being of an antitoxic nature. The neutralization of the pollen poison—so far as it occurs at all—does not follow the law of proportions applying to the neutralization of toxins and antitoxins, according to which the

* The author has had excellent results in a number of cases of hay-fever with vaccination-therapy. The vaccine is prepared by macerating rag-weed pollen in a solution of 1 : 100,000, 1 : 10,000, etc. The sensitiveness to pollen of the individual is determined by *intracutaneous* injection, and the treatment begun with subcutaneous injections of the minimum dose to which reaction has occurred, the dose being slowly increased from week to week. No untoward symptoms have as yet been observed, but in several cases the hay-fever symptoms have continued. The treatment, therefore, can hardly be recommended as yet, and we must await more extensive experiments before we shall be in a position to pass judgment upon it.

double amount of poison must be saturated by the double amount of antitoxin.

A curve of saturation published by Dunbar's own school shows distinctly the truth of the above statement. After this contradiction was pointed out, a curve of saturation was published corresponding to the law of proportion. Anyone can see for himself the objective incorrectness of the latter by performing experiments in saturations in hay-fever with native unaltered pollen. He may also prove that pollantin is not an antitoxin by injecting pollantin, together with pollen poison, into a person sensitive to the latter—an experiment which it would be well to try only on one who is convinced of the antitoxic nature of pollantin.

Pollantin is a lytic serum, as is proved by the experiments of Dunbar himself, who showed by complement-fixation methods that pollantin contained amboceptors ('Bericht 11, Heufieberbundes,' p. 6).

In all probability the action of both sera depends upon colloidal inhibitory substances present in the serum.

Intermediate Cases between Healthy Individuals and those Sensitive to Pollen.—In contrast with hay-fever victims are those healthy individuals who do not reabsorb pollen albumen. If, however, a strict line could be drawn between hay-fever patients and normal individuals, it would be impossible to explain how pollen sensitiveness is acquired. As it is, the author has shown by experiments that between these individuals not reacting to pollen and those extremely sensitive to it there are a great many people who show subjective pollen reaction (itching) upon the local application of pollen, without exhibiting objective symptoms at the conjunctiva and nose. We even find cases where persons who do not suffer from hay-fever show an objective reaction to pollen albumen. There are also many degrees of pollen sensitiveness among true hay-fever patients, some reacting distinctly to one drop of pollen emulsion of 1 : 10,000, while others react only to a dilution of 1 : 50.

Thus there are many degrees of pollen sensitiveness and insensitiveness, which explains how these conditions may gradually develop and become stronger in an individual.

Hay-fever may be considered analogous to urticaria, although it apparently offers an entirely different symptom-complex.

Urticaria.

According to what has just been stated, a number of comparisons may be drawn between hay-fever and urticaria. It is interesting to note that physicians of the old school, whose observations along clinical lines still call forth our admiration, had noted the relation between hay-fever and urticaria. They mention the fact that in families in which hay-fever occurred there was frequently a tendency to urticaria, and, further, that hay-fever and urticaria occurred vicariously even in the same individual. Another interesting fact in this connection is that Dunbar has observed the abrupt onset of urticarial eruptions after subcutaneous injections in pollen-sensitive individuals.

The Nature of Idiosyncrasy.—These discoveries supply us for the first time with a clue to the true nature of idiosyncrasy. For this reason they are also of great clinical significance. 'Idiosyncrasy' was formerly a word without meaning, a means of hiding the ignorance of physicians as to the real conditions, a placebo, serving the same purpose as did for years the term 'rheumatoid.' *A great many idiosyncrasies can now be explained as hypersensitiveness toward the ingestion of heterogeneous albumen. This hypersensitiveness becomes especially manifest whenever injections of albuminous substances have previously been made.* The incorporation of heterogeneous albumen produces phenomena, among which urticaria, erythema, and other cutaneous affections, represent the relatively least harmful forms.

These facts will solve a number of clinical problems, particularly that of the etiology of urticaria.

Various Etiologies of Urticaria.—In the first place, the reabsorption of heterogeneous albuminous substance offers a reasonable and, what is far more important, a consistent explanation of a great variety of urticarial eruptions—viz., that from nettles; the sting of bees or wasps; the bite of mosquitoes, gnats, etc.; and, above all, the eruption following

the rupture of an echinococcic cyst of the liver, whereby the contents of the cyst have been emptied into the peritoneum, and a subsequent reabsorption has taken place—an urticaria which was formerly looked upon as unexplainable and unique.

Urticaria ex Ingestis.—Cases in which urticaria originates in the intestines, commonly known as 'urticaria ex ingestis,' demand special attention. In these cases we have to deal with the ingestion of heterogeneous albumen, which is not as a rule introduced subcutaneously, but *per os*. We have stated that in the majority of cases of urticaria ex ingestis intestinal disorders as well as an increase of indican in the urine are present. L. Michaelis has demonstrated that in normal intestinal digestion albumen is deprived of its specificity. It is very probable that in individuals affected with urticarial disorders the albumen is not split up by the intestinal secretions sufficiently to lose its identity, so that particles of heterogeneous albumen are absorbed into the circulation. According to well-known laws, no insensitiveness ever follows even a continual reabsorption of heterogeneous albumen; on the contrary, a hypersensitiveness may result. Thus it is clear why certain individuals are affected with urticaria whenever they ingest that particular albumen upon which their intestinal juices act insufficiently. This peculiarity disappears only when the intestinal juices split up the substances to such an extent that their specificity is entirely lost, and any urticarial eruption, of course, precluded. Thus persons who in childhood suffered from urticaria after indulging in certain foods often find the same foods agreeing with them in later years.

Urticaria due to Drugs.—At first thought it would seem more difficult to explain those cases of urticaria following the administration of drugs, and presenting the same clinical symptoms as serum exanthema. The researches of Obermayer and Pick have demonstrated that albuminous substances originating from the same species of animal are transformed from native albumen into heterogeneous albumen by treatment with iodides, nitrites, or diazo (*Wien. Klin. Woch.*, 1906). The albumen so denaturalized produces the same

reactions in the animal's body as does heterogeneous albumen when injected from the first—*e.g.*, precipitin formation. Hence, according to these researches, there is an etiology for drug exanthema analogous to that of the other forms of urticaria, if we may assume a fixation of the albuminous molecule to the drug. However, considering the fact that Mibelli, Raviart, and Thoney have proved the existence of antipyrin in skin blisters, and that the same phenomena follow the external application of this drug to antipyrin-sensitive individuals, it would seem possible that the blisters are due to a direct action of this drug. In this case these drug exanthemata would probably not come under the category of exanthema caused by heterogeneous albumen.

Urticaria of Constipation and Pregnancy.—The urticaria of constipation and pregnancy finds a very simple explanation in the above-defined law of sensitiveness to albumen.

Urticaria as a whole would, therefore, constitute a syndrome with a uniform etiology. However, there are still cases that are somewhat difficult to classify. For example, there is the urticaria of menstruation, which is, to be sure, very rare. It must be remembered that processes take place in menstruation about which little was known until recently, and which were ascribed to internal secretion. In these processes it is very probable that an alteration of albumen is taking place, giving rise to an urticarial eruption. We know how slight a disturbance of the albuminous molecule is needed to show properties of an entirely different nature—*e.g.*, Weichardt's toxins of fatigue and reduction.

According to what has just been said, urticaria may be classed in two general groups—viz.:

1. Cases in which the urticaria is caused by the introduction of heterogeneous albumen from without.

2. Cases in which the poison originates directly or indirectly from the body itself.

Strictly speaking, cases of urticaria ex ingestis would belong to the first group; yet the poison originates indirectly from the body itself, since, according to Obermayer, Pick, Michaelis, and Oppenheimer, the intestinal canal in animals possessing such, and therefore taking up nourishment directly,

must so split up the heterogeneous albumen that synthesis to body albumen may take place.

Some problems, and with them material for further investigation, are yet found in the etiology of urticaria. If urticaria is attributable to the reabsorption of heterogeneous albumen, as seems to be well established, naturally this albumen may be organized or unorganized—in other words, the question is not yet settled as to whether urticaria is caused by living excitants. Yet this assumption fails to apply to a great many cases.

Central Production of Urticaria.—Although Lesser claims that, according to his experiments in the etiology of urticaria, there is a central action from the brain, this is in no wise inconsistent with the views of Wolff-Eisner, who has always held that there was an action of toxins and endotoxins originating centrally, and according to whose experiments in heterogeneous albuminous substances only that agent was found which, *in the great majority of cases*, causes the irritation of the centres. It is not necessary to repeat that the centres may be irritated in other ways—as, for example, in blushing (erythema fugax)—when we consider that muscular contractions may also be produced through the nerves by numerous forms of irritation.

In this way there is a relationship between urticaria and various pathological conditions of the intestines (pedatrophia, etc.), as clinicians have often suspected. To illustrate: The author recently observed a case of nephritis, accompanied with intestinal trouble, in which a severe urticaria seemed to indicate a reabsorption of toxic heterogeneous albuminous substances, and in which a regulation of the function of the skin by dieting resulted in a decided improvement of the nephritis, and—what is far more important in judging the syndrome—a disappearance of the urticaria. Careful attention is now being given by dermatologists to this relationship between the reabsorption of heterogeneous albumen and urticaria, and the importance of these processes is being emphasized.

Bruck has in various works produced evidence supporting this view of urticaria, showing that while the tendency to

illness after indulgence in crabs and the like cannot be transmitted with serum, typical symptoms of hypersensitiveness may be transmitted. However, all investigators have not reached the same results, so that the possibility of the transmissibility of yet other factors (individual sensitiveness) is still in doubt.

Pellagra.

Pellagra belongs to this group of pathological conditions—at least, in its first stage, which begins with indefinite prodromal symptoms. In the spring an erythema appears on the dorsum of hands and feet; then the disease apparently disappears. In the second stage the same phenomena appear in a more intense form, and the symptom-complex is complicated with gastric disorders and diarrhœas. Muscular atrophy and nervous symptoms occur as early as the second stage. These lead to the third stage, which is complicated by psychic symptoms. Death finally ensues in cachexia. This disease is ascribed to a continued use of decayed Indian corn, which, by reason of certain conditions existing among the very poor, is harvested when over-ripe and moist, or, as Joseph claims, unripe.

Eclampsia.

All attempts at demonstrating a specific poison in the placenta of eclamptic women must be regarded as complete failures. It is almost impossible to avoid bacterial contamination in these experiments, and the alterations produced by the injection of eclamptic placentas have not been at all characteristic of the symptom-complex and the pathologic-anatomical picture of eclampsia. The entire course of eclampsia, with convulsions, nephritis, hæmorrhagic diathesis, and the fact that it is influenced by narcosis, is strikingly similar to the syndrome produced by the repeated reabsorption of heterogeneous albumen. The placenta in the uterus of a pregnant woman is only partially a product of the ovum, and heterogeneous albumen is also represented (proportion of spermatozoon). It is not irrelevant to mention in this connection that the high degree of toxicity of the spermatozoon has been ascertained by

researches of the author. It is not a mere probability that a reabsorption of placental villi occurs repeatedly and frequently, but it has been demonstrated by the pathologic-anatomical researches of Schmorl and others.*

Weichardt was the first to point out the significance of the lytic processes from the serologic standpoint, and he has produced passive eclamptic symptoms by means of the serum of animals treated with placenta. Rosenau's opinion that the question of eclampsia concerned the repeated absorption of villous elements is more in agreement with the clinical symptom-complex, the pathologic findings, and the experiences of modern research in immunity than is any other theory.†

We now know positively that the heterogeneous albumen from the syncytium finds its way into the circulation of the pregnant woman, and under certain conditions eclampsia ensues as a sign of the reabsorption of albumen and the resulting hypersensitiveness. The reason, it was difficult to understand the conditions, was, that eclampsia was a comparatively uncommon disease. Now, however, as in hay-fever, we can ascribe a number of disorders in pregnancy to the reabsorption of heterogeneous albumen (urticaria, albuminuria, perhaps even the vomiting of pregnancy, and eclampsia). Hence eclampsia would appear to be merely the severest and rarest form of phenomena resulting from the reabsorption of heterogeneous albumen. The various clinical manifestations of the reabsorption of heterogeneous albumen during pregnancy would, therefore, correspond

* The exact proof of the reabsorption of these heterogeneous albumens is convincingly shown by Gräfenberg, of the gynæcological clinic of Kiel. 'In the first three months the placentar villus shows heterolysis—*i.e.*, it acts digestively on the serum plate. The purpose of this process is perhaps to facilitate the imbedding of the ovum. The fact that during pregnancy, probably as a reaction from the reabsorption of tryptic ferments from the chorion, the amount of antitrypsin in the maternal serum increases to double the normal content is to be regarded as a sign of the reabsorption of parts of the chorion by the maternal organism' (*Münch. Med. Woch.*, 1909, No. 14).

† Kraus attributes the cutaneous symptoms in syphilis and typhoid fever to the proliferation of isolated micro-organisms in the capillaries (*Wien. Klin. Woch.*, 1907, No. 9).

roughly to the widely varying clinical symptoms following the resorption of pollen albumen, as, for example, harmless conjunctivitis on the one hand and severe asthmatic attacks on the other, both caused by resorption of the same albuminous pollen substance.

Vaccination and Revaccination.

A symptom-complex similar in many respects to that of serum disease, yet very different, is to be had in the phenomena of vaccination and revaccination. The clinical significance of these phenomena is apparently very slight. The question concerns first of all a virus capable of proliferation —the substance producing the phenomenon—in contrast with the serum.

In the symptom-complex of revaccination it is very evident that the altered condition of the body, formerly regarded as an indication of immunity, is *merely a manifestation of an accelerated reaction.**

Course and Time of Reaction in Revaccination.—In the first vaccination the acme of the vaccination process occurs usually on the ninth, twelfth, or fourteenth day. In revaccination the period of reaction is shortened, and the process usually runs a course without fever. If a revaccination is performed shortly after vaccination, an immediate reaction occurs, which becomes merely an 'accelerated reaction' if a longer interval is allowed to elapse.

An interesting fact reported by Pirquet is that in successive vaccinations of an individual made on different days the

* *Cf.* Wolff-Eisner, *Berl. Klin. Woch.*, 1904, Nos. 42-44, p. 12: 'The animal previously treated shows, in contrast with the control animal, the property of a speedier lysis.' *Zentr. f. Bakt.*, 1904, vol. xxxvii., No. 5, p. 684: 'The morphological proof is given that lysis is accelerated in the immune animal.' Pirquet (1905): 'The nature of clinical immunity for this group of diseases, which we regard as analogous to vaccination, does not consist in an insensitiveness acquired against the infection exciter, but in the capacity for an accelerated reaction' ('Serumkrankheit,' p. 135). Again (1905): 'The accelerated reaction is the permanent advantage acquired by the organism in overcoming the first disease' ('Serumkrankheit,' p. 134).

The first author not only illustrates the fact of an accelerated reaction in immunity, but points out the cause of the phenomenon. It was necessary to make sure of this in view of Pirquet's presumptuous statements (*Berl. Klin. Woch.*, 1908).

acme of all the areolæ occurs with that of the first vaccination—*i.e.*, when the vaccination of the longest duration shows an areolar formation.

It was pointed out in the Introduction that Pirquet described the clinical symptoms of hypersensitiveness rather than the phenomena, resulting in death, which were worked out by Wolff-Eisner. But even in revaccination the great *clinical* significance of this second form of hypersensitiveness (showing severe clinical reactions, and sometimes resulting in death) is shown so clearly that the conclusions which Wolff-Eisner drew in 1904 were drawn almost identically in 1905 by Pirquet from his experiments. Both works are cited in the footnote on p. 66.

In revaccination a very severe reaction sometimes appears, designated by Pirquet as a 'hyperergic' reaction. There is another form of variola of considerable significance, an especially malignant form, known as 'purpura variolosa,' occurring only in *vaccinated persons*. There is an especially short incubation period in these cases, and the affection results in death, with the symptoms of a hæmorrhagic diathesis. This symptom-complex cannot be designated otherwise than as a hypersensitiveness (in the sense of Wolff-Eisner's severe reactions), leading to a speedy death, with the symptoms described.

Syphilis.

Herxheimer's Reaction.—Far too little attention is paid in syphilis to the reaction discovered by Herxheimer. This reaction depends upon the fact that at the beginning of a mercurial treatment a considerable increase of the exanthema takes place, or an exanthema appears which was previously absent. Among all the explanations given of this phenomena the most probable is that the exciters of syphilis (spirochætæ) are killed by mercury, but that they have, nevertheless, induced an exanthema.

Thalmann* was the first to treat in detail the signifi-

* *Münch. Med. Woch.*, 1908, and 'Medizinische Abteilung des Sachs Kriegministeriums,' 'Die Syphilis und ihre Behandlung in Lichte der neueren Forschungen,' Dresden, 1906.

cance of endotoxins in syphilis. This was done, however, without a sufficient knowledge of the literature then to be had on the subject. His conclusions, which are of considerable importance, may be briefly stated :

Endotoxins in Syphilis Research.—1. Mercury is intensely bactericidal to spirochætæ.

2. The poisons incorporated in their bodies—*i.e.*, *syphilis endotoxins*—are thereby liberated. These cause hyperæmia, round-cell infiltration, tissue hypertrophy, and formation of new tissue.

By this liberation of poisons through the death of spirochætæ an exacerbation takes place during the first few days of mercurial treatment (Herxheimer's reaction).

3. Early in the disease, especially after the death of the spirochætæ from Hg, and the consequent liberation of quantities of endotoxins, there is a corresponding abundant formation of antibodies, and with it an increase of immunity to the poison. This, after an exanthema lasting for weeks, may be so considerable that even without treatment an arrest and improvement of the disease may result.

4. The living spirochætæ gradually continue to proliferate, and immunity decreases. Fewer foci are now present. Roseola with large spots now appear, for there is a longer interval before a high degree of immunity recurs. Further relapses are explained in this way.

5. Later the number of foci decreases. The foci contain smaller and much weakened spirochætæ ; consequently, but few antibodies. However, a certain degree of immunity still holds from the previous condition.

6. The endotoxins have very considerably weakened the body cells. In the tertiary stage very few spirochætæ are present. Now, however, very slight amounts of poison are sufficient to cause a destruction of the tissue and to produce ulcerative processes.

7. Malignant syphilis is that in which the body is incapable of producing antibodies.

8. The later metasyphilitic affections consist in a premature cellular degeneration, arising very easily through the weakening of the cells, caused by incidental factors (alcohol, infectious diseases, overexertion, traumatism, etc.).

Equivalents of Herxheimer's Syphilis Reaction.—The so-called Herxheimer reaction, which, according to Thalmann, has already been described by other authors, and which consists

in the intensification of roseola after mercurial treatment—the stronger the action of the mercury, the more intense the roseola—is in Thalmann's opinion equivalent to the aggravation of tonsillary affections, to the increase of hoarseness in syphilitic laryngitis, and to the appearance of iritis and headache after mercurial treatment.

We may accept unconditionally most of Thalmann's statements. We may add that Thalmann ascribes the phenomena to the quantitative proportions of the antibodies (reactive substances) only, without taking into consideration the hypersensitiveness arising in the course of the disease. Hypersensitiveness explains the fact that even small amounts of virus are sufficient to bring about severe clinical alterations. In malignant syphilis there is certainly no lack of ability to produce antibodies; on the contrary, there is present an abnormal reaction to the virulent bodies liberated by the lysis of the spirochæte. The author has observed such a reaction to trypanosomata of rats. The positive results of the Wassermann reaction show that in tertiary syphilis also the cause of the severe clinical phenomena is not to be found in an absence of antibodies.

Thalmann's views have greatly enhanced our knowledge of the nature of syphilis, and the trivial differences in opinions do not alter the matter. Other points still open to discussion are as follows: In spontaneous healing of syphilis it is sufficient to assume that antibodies destroy the spirochætæ; the clinical phenomena do not necessarily indicate the presence of antitoxic substances. In explaining the tertiary symptoms we need not assume, with Thalmann, a specific weakening of the body cells by syphilitic endotoxins, since as a rule after a repeated absorption of a heterogeneous albumen a *hypersensitiveness* may be developed, in consequence of which *small amounts of this substance* (*i.e.*, a very few spirochætæ) *may elicit more intense, though sometimes merely local, symptoms.* It may easily be proved by experiments that Thalmann's opinion in statement 7 (p. 68) as to the absence of antibodies in malignant syphilis is incorrect. If Thalmann's views were right, there should be an incubation period of from four to six weeks in malignant syphilis, after rein-

fection with spirochætæ, before the appearance of papules —primary lesion of reinfection.*

The fact that syphilitics are not reinfected by specific material applied to the skin, Thalmann still ascribes to 'immunity.'

Experiments in Reinfection with Syphilis.—The experiments of Finger, Landsteiner, Neisser, and Wolff-Eisner prove that this is not correct, and that, on the contrary, the question is one of an accelerated reaction in the form of a hypersensitiveness, as in revaccination and the tuberculin reaction.

The term 'dosis letalis minima' introduced by the author for the purpose of establishing the limits of bactericidal serum-therapy (*Berl. Klin. Woch.*, Nos. 17 and 20, 1903) was adopted and employed by Thalmann in discussing the theory of syphilis. Wolff-Eisner had laid emphasis upon the fact that small amounts of endotoxin act in a positive chemotactic, larger amounts in a negative chemotactic, manner. This was modified by Thalmann to read: 'Endotoxins in small quantities form a stimulus to cell-division and new formation; in increased concentration or in a more intense virulence they lead to necrosis. . . . The individual tissues act in different ways: the stimulus which acts upon connective-tissue cells as a plastic irritant produces a degeneration in sensitive nerve cells.' The same laws hold good for the action of tuberculin in the formation of tubercles and the transition to caseation. By comparing Thalmann's view with what we know of symptoms occurring after a repeated reabsorption of heterogeneous albumen, we find that the syphilitic exanthema is caused not by the spirochætæ themselves, but by the substances liberated through their lysis. Consequently, without therapeutic interference an exanthema arises only in case the reactive substances have been produced in a sufficient amount, exactly as with serum exanthema. The question has long been agitated in the medical world as to whether a syphilitic could be infected a second time, and whether it was possible to produce a new primary lesion in a syphilis already existing. The researches

* Neisser, *Berlin. Med. Ges.*, 1908.

of Finger, Landsteiner, and others, show that this is possible. The author's experiments attested that the claim that a reinfection in syphilis was impossible could not be maintained, much less that an absolute immunity existed. Reinfection has, like revaccination, a shortened incubation period (usually only twenty-four to forty-eight hours), and this period of incubation does not seem to vary in the different stages of syphilis.

The new primary lesion arising in reinfection is usually a small papule with an abortive course. As Wolff-Eisner's findings show, another form of hypersensitiveness, though rare, may occur, manifested by severe clinical symptoms, but not running a rapid course. In reinfection an ulcerous gumma next appears. Further experiences will show whether there really is a relationship between these symptoms and malignancy of syphilis, as Wolff-Eisner suspected.

The view, held for a long time, that the increase of bacteria resulted in death from purely mechanical reasons is incorrect. This is proved not only by the fact that an increase of bacteria takes place only during the last hours, when the fate of the animal is already decided, but is most clearly shown in rat trypanosomiasis. Here trypanosomata may often exist in enormous quantities without in the least affecting the general condition of the rat, although the extremely motile trypanosoma is much more capable of inflicting mechanical injuries than most forms of bacteria. This fact cannot be explained except by assuming that there is a symbiosis between the rat and trypanosomata of such a nature that the rat's body does not attempt to free itself from the reactive substances of the trypanosomata. Thus only those trypanosomata which die a natural death are broken down.*

* In this connection it is of especial interest to note that in transferring rats' blood containing trypanosomata to other test animals (mice, guinea-pigs, or rabbits), the trypanosomata proliferated for a time, without causing any special symptoms. After the injection of a moderately high dose, however, some of the guinea-pigs died, and in these animals no trypanosoma could be found in the blood and organs, while in the other animals infected at the same time large numbers of trypanosomes were present. It is reasonable to believe that with the killing of the trypanosomes poisons were liberated which killed just those animals which attempted to rid themselves of infection by lysis of the trypanosomes.

Pneumonia.

Rôle of Bacteriolysis in Crisis and Pseudo-Crisis.—In pneumonia the fever results from the lysis of pneumococci. To understand crisis and pseudo-crisis it is necessary to remember that small and moderate amounts of endotoxin raise the temperature and large amounts lower it, as may easily be seen in experimental typhoid and cholera infections. The bacteriolysins present in the body cause a continuous lysis of pneumococci, and the endotoxins liberated at the same time cause the fever. By the irritation elicited through the toxins liberated by the bacteriolysis the same antibodies are now formed in increased quantities. It is some time before these appear in the circulation (incubation period of antibody formation).*

The infection can terminate only after the activation of these antibodies, and this may have been anticipated by the observations of old-school physicians who considered the odd days (fifth and seventh) associated with the crisis of pneumonia. As soon as a larger number of lysins has been formed an intense lysis occurs, accompanied by perspiration, fall of temperature, increased pulse, etc. If this lysin-antibody attack results in the destruction of all the pneumococci, the disease is terminated and the crisis is past. If, however, pneumococci still survive, the termination is decided by a new supply of antibodies; yet even then we may have another crisis, or a pseudo-crisis. Thus we cannot tell by a lowering of the temperature whether a crisis or a pseudo-

* One of Pirquet's observations makes this process much easier to understand. Pirquet demonstrated that vaccinations (with cow lymph) made on seven successive days showed results the same day (see 'Vaccination'). The vaccinations become positive simultaneously because the antibodies (lysins) which are formed neutralize all the virus that is present, and the incubation ends when the lysins are formed in a sufficient amount. The processes in the crisis of pneumonia are very similar. It would be wrong to assume that antibodies accumulate before they begin the attack; they begin the attack as soon as they are formed. The formation, however, is stimulated when, owing to the irritation of the endotoxins liberated by the bacteriolysins spontaneously present, they are cast off from the cells, particularly, it would seem, in the hæmatogenic organs.

crisis is at hand, as the clinical symptoms of both and their causes are exactly the same.*

For antibodies in pneumonia, see Appendix.

Typhoid Fever.

The experimental typhoid infection in animals runs a fatal course in twenty to thirty hours. As typhoid fever in human beings is an infectious disease of long duration, localized chiefly in the intestine, it was formerly believed that there was no relation whatever between the infection in animals and that in man, and that the character of typhoid symptoms was dependent upon the intestinal affection. Meanwhile a series of typhoid cases was observed with slight or no intestinal affection, and both Stadelmann and Wolff-Eisner ascribed the symptoms of typhoid fever to the action of typhoid endotoxins, as such cases may run a course clinically analogous to *typhus abdominalis* (fever curve, etc.).

Typhoid Fever as an Endotoxin Affection.†—If intestinal ulcers and localization of the disease in the intestines do not constitute the syndrome of typhoid fever, the affection is an intoxication caused by the dissolved bacterial bodies (endotoxins). This view is also advocated by Neufeld. *Taking this ground, it is only logical to believe that the protective forces of the body—the bacteriolysins—produce the symptom-complex.*

In the typhoid patient these lysins are present in quantities too great to permit typhoid bacilli to remain undissolved in the blood, as in the urine and bile. On the other hand, they are insufficient to destroy all the typhoid bacilli quickly by bacteriolysis. In a work on research published in 1903 ('Investigations in a Few Questions of Immunity,' *Berl. Klin. Woch.*, 1904, Nos. 42-44; and 'Fundamental Laws of Im-

* Romberg has pointed out that the phenomena of pneumonia are not attributable to interference with the respiration, but to poison especially affecting the heart.

† *Cf.* Stadelmann, and Wolff-Eisner, *Munch. Med. Woch.*, 1907, Nos. 24 and 25.

munity,' *Centr. f. Bakt.*, vol. xxxvii., Nos. 3-5), the authors very emphatically set forth the view that in certain cases a bacteriolytic serum might cause death. It is difficult to understand now why this view should have met with so much opposition, when it merely meant a consistent application of the experiences of bacteriolysis to clinical problems.*

Typhoid Relapses.—Relapses in typhoid fever made it practically impossible to explain the clinical symptoms of typhoid in any other way. As Jürgens points out (*Deutsch. Med. Woch.*, 1907), it is unsatisfactory to assume that relapses are dependent upon a new proliferation of bacteria in the intestines. Scholastic medicine assumed that after overcoming an attack of typhoid fever an immunity developed. This was regarded as proved experimentally by agglutinins and bacteriolytic immune bodies. There is no doubt whatever that such 'reaction bodies' (as they should be called, rather than 'immune bodies,' since the latter term implies nothing) are formed; but they do not afford a certain protection against a new infection or against a relapse. Jürgens had already reported some cases in which, in spite of a high bactericidal titer of the blood-serum, relapses occurred. He also considers the assumption of an immunity pure nonsense.

He is right and yet wrong. In this case it would be better not to call the phenomenon immunity. Nevertheless, the bacteriolytic immune bodies play a part if in the majority of cases there is no relapse and no reinfection, even when typhoid bacilli are still present in the organism. *The cause of this apparent inconsistency is the uncertain action of the bacteriolytic immune bodies, inasmuch as the same bodies may cause death*

* Since this explanation includes all typhoid cases, it is of no especial importance whether or not we differentiate cases without intestinal localization, such as spleno-typhoid (Eiselt, Verh. des X Intern. Med. Kongr., part 2, vol. v., p. 210, 1891) or, like Curschmann, apply the term 'toxin-typhoid.' We call attention to the importance of typhoid sepsis in order to show that typhoid infection may run the same course in man as in animals. On the other hand, Bail claims that after an intravenous injection in rabbits of typhoid bacilli or aggressin there may be intestinal alterations similar to those in human typhoid, and, therefore, typhoid fever may run the same course in animals as in human beings (*Wien. Klin. Woch.*, 1907, No. 10).

or recovery, according to the amount of bacterial toxins liberated by bacteriolysis (endotoxins).

The bacteriolysins of an organism that has overcome a typhoid infection are generally sufficient to act before endotoxins have been formed in any considerable amount. If the bacteria succeed in proliferating—in places, perchance, protected from bacteriolysis—the high bactericidal content may become dangerous to life, provided the bacteria invade the blood.

Under these circumstances we cannot expect much from the serum-therapy of typhoid fever.

Typhoid Infection in Human Beings and Animals.—The question now arises whether the different syndrome in animal and man can be explained fully by the action of the endotoxin. For this explanation we must again refer to the fundamental law that small doses of endotoxin cause a rise and large doses a fall of temperature. If we keep this fact in mind, it is at once evident why in typhoid fever in man we have a series of increased temperatures, but in guinea-pigs, following a short rise in temperature, there is a decrease lasting until death. In human typhoid infections small amounts of typhoid endotoxin are gradually carried into the circulation, while the organism of the guinea-pig in peritoneal infection is flooded with typhoid endotoxin; moreover, bacteriolysis is more intense in the organism of the guinea-pig than in human serum. This is the real and essential difference between human and animal infection with typhoid bacilli. Assuming the same susceptibility to endotoxin, bacteriolysis in guinea-pigs is acute; in man, subacute. For this reason the symptoms in guinea-pigs are more severe, and it is manifest earlier in the disease whether or not the termination will be fatal.

The more rapid and intense bacteriolysis in guinea-pigs also explains why typhoid infection through the intestine, though usual in man, does not occur in guinea-pigs. The bacteria entering the intestines through the circulation are bacteriolyzed, and do not find hiding-places, like those in human beings, where they may establish themselves and proliferate, protected from bacteriolytic action. The fact that

human beings, like guinea-pigs, are not necessarily affected by typhoid infection from the intestines is so well known that it needs no repetition. As a matter of criticism of Metchnikoff's phagocytic theory, it might be interesting to know whether the typhoid bacilli find hiding-places in the spleen or in the lymph glands. It would be interesting to clinicians and therapeutists if these places could be found. Our present technique, however, only permits us to suspect that typhoid bacilli may hide and proliferate in the spleen and lymph glands undisturbed by the bacteriolytic forces of the serum.

Incubation in Typhoid Fever.—Jürgens (*Deutsch. Med. Woch.*, 1907, No. 1) reports that weeks, months, or even years, may elapse between the ingestion of typhoid bacilli and the appearance of clinical symptoms. He styles this interval an 'incubation period'—not quite correctly, it would seem, since the term 'incubation,' in the sense in which it is used by clinicians and bacteriologists, has a different meaning. By 'incubation' we understand, as does Jürgens, the interval between the infection and the appearance of the disease, but with the proviso that in the meantime the specific organism has proliferated. If the proliferation has advanced so far that the bacteria may produce symptoms, the incubation is at an end.

To interpret the phenomena of incubation we must remember that the proliferation of the bacteria takes place largely during a continuous struggle with the bactericidal forces of the body. We must assume that incubation terminates when the amount of endotoxins freed by bacteriolysis reaches the *dosis efficax*, or, after passing the fixed receptors, arrives centrally at the vital organs (brain, etc.).

The cases presented by Jürgens, in which there was an interval of some months between the infection and the appearance of the disease, had to do with an incubation like that above mentioned, as the disease-producing organisms proliferated and accumulated uninfluenced by the bactericidal forces of the body. Symptom-complexes can occur only when bacteria for some reason meet with the bactericidal power.

The adversaries of modern epidemiology—*e.g.*, Rosenbach —have always pointed out that in typhoid infection of guinea-pigs we have to deal, not with an infectious disease, but with an injection disease, since the course of the disease in guinea-pigs does not show the slightest similarity to that in man. We may consider that we have made a great stride forward if we are able to explain animal and human infections, with their great clinical differences, by the same conception, and to ascribe these differences to a mere *quantitative* difference in bacteriolytic immunity.

Tuberculosis.

Influence of Endotoxins on the Clinical Symptoms of Tuberculosis.—The symptoms of tuberculosis, such as fever, night-sweats, fluctuation of temperature, and loss of weight, are to be attributed to poisons (endotoxins) liberated by the lysis of tubercle bacilli. In case tuberculosis is already present, a reinfection has an accelerated and abortive course similar to that of syphilis. The only exception to this is in reinfections with massive infectious doses (Römer).

Symptoms similar to those of tuberculosis of spontaneous origin can be produced by artificial incorporation of tubercle bacilli derivatives (tuberculin). Tuberculosis is peculiar in that tubercle bacilli strongly resist lysis. This explains the long incubation after infection with tubercle bacilli. The absence of lysins further explains why normal individuals—*i.e.*, men or animals free from tuberculous infection—are absolutely uninfluenced by dead tubercle bacilli and tuberculin (Schreiber, Hamburger, Erlandsen, and Wolff-Eisner). As a rule, only those individuals who are infected with tuberculosis or who have withstood such infection possess substances eliciting a reaction and liberating poisons when brought in contact with tuberculin or tubercle bacilli products.*

* Experiments of the author have proved beyond question that it is possible, even with healthy animals (rabbits), to produce experimentally hypersensitiveness to derivatives of tubercle bacilli by long-continued treatment, particularly with ground tubercle bacilli or antibodies—*i.e.*, reactive substances which elicit a reaction when in contact with tuberculin or

Various Forms of Tuberculin Action.—The various reactions, depending upon the kind of tuberculous infection or the time that has elapsed since infection, are as follows:

(*a*) Immediate reaction, if a copious amount of lysin antibodies is present.

(*b*) A delayed reaction, if only small amounts of lysin are present.

(*c*) Reaction only after reinjections, if the formation of lysin has been elicited by the stimulus of the first injection, the injections here stimulating first the capacity of the body for producing reactive substances (antibodies), the presence of which becomes manifest after a new injection of tuberculin.

For a further discussion of tuberculin diagnosis, see p. 129.

Conclusions as to the Nature of Immunity, drawn from the Syndrome dependent upon Hypersensitiveness.

Antigen Theories in Hypersensitiveness.—In the foregoing a number of experimental observations and various symptom-syndrome are introduced, attesting that not all symptoms of infectious disease are to be ascribed to the living bacteria themselves, but merely to the poisons (endotoxins) freed by lysis. Since in 1904 a complete analogy was drawn by the author between endotoxins and heterogeneous albumens,* all the albumen-diseases thus far mentioned are only special instances of the great fundamental law governing infectious diseases. In this way a peculiar relationship is shown between the syndrome and the antibodies regarded as protective substances.

Wolff-Eisner (*Berl. Klin. Woch.*, 1904, Nos. 42-44) expresses this idea very clearly.

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derivatives of tubercle bacilli. Yet, aside from experimental measures of this sort, it is safe to assert that anyone who has not been infected with tuberculosis does not react to tuberculin.

* *Berl. Klin. Woch.*, 1904, Nos. 42-44, pp. 6-15; *Centr. f. Bakt.*, 1904, vol. xxxvii., p. 687: 'The bacterial endotoxins do not constitute a special group of poisons, with their own laws, but are a heterogeneous albumen, poisonous as are all heterogeneous albumens.'

'In bacterial immunity the lysins *appear* to protect the animal against endotoxins. However, in reality they only inhibit proliferation at a time when the amount of endotoxins has not yet reached the minimum fatal dose.'

'The fate of the animal is determined by the endotoxins freed by bacteriolysis.

'A bacterial serum may be of very great assistance in saving life in a bacterial infection by inducing a bacteriolysis which destroys the infecting micro-organism.

'If, however, the fatal dose of endotoxins has been exceeded, the bacteriolysis induced by the immune serum will only result in hastening the fatal termination, or in some cases will even cause it.'

It has been made clear in the citations above that these antibodies are by no means identical with antitoxins, but represent anti-substances of a very different kind. This view is not nearly so well emphasized in Pirquet's articles, in which there is an evident tendency to identify antibodies with antitoxins.

In 1903 Pirquet ('Theory of Incubation,' *Wien. Klin. Woch.*, 1903, No. 45; and 'Ges. f. Kinderheilk.,' Cassel, 1903) ascribed incubation and the phenomena of revaccination to antibodies, but always to antibodies uniting with antigen, and thus producing reaction. He says, for example: 'A fundamental law of antibody formation is that these are produced more rapidly and intensely after a repeated action of the stimulus.' Since the term 'antibodies' was generally understood to mean antitoxins, Pirquet's theory met with the objection that antitoxin formation in diphtheria produced no pathological symptoms. However, Pirquet placed no emphasis upon the difference between these antibodies and antitoxins, and the *lytic* character of the antisubstances referred to by him, but, becoming dualistic, declared that *other* antibodies produced the disease symptoms, and still others terminated the disease.

Pirquet in 1905 said: 'As for the serum, we were able to demonstrate that it did not in itself act as a toxin, but that the toxic body was produced only by an interaction of organism and antigen.

'The view that antibodies, which are supposed to afford protection against a disease, may cause the disease seems absurd at first thought. However, this is because we are accustomed to see in disease only an injury to the organism, and in antibodies merely antitoxic substances ('Serumkrankheit,' p. 129).

Even here Pirquet has not entirely given up the idea that antibodies are of antitoxic character. For instance, he regarded the accelerated reaction occurring after the reinjection of serum as, strictly speaking, a phenomenon of immunity, 'because the phenomena subside more rapidly, and in spite of its sensitiveness, the organism *in toto* is injured less than by the first injection' (*Wien. Klin. Woch.*, 1905, No. 17). Thus, even as late as 1905 there was a complete misinterpretation on the part of Pirquet of the nature of hypersensitiveness, which the author met by pointing out the lytic nature of these antibodies. (*Centr. f. Bakt.*, vol. xl., No. 3).

It is evident from the arguments of these authors (Pirquet and Schick) that they assume some kind of immunity at the onset of serum disease. In reality, however, in the injection of organic albumen, which the author used in his experiments, the matter stands as follows: After a repeated injection there occurs not only a *hastened, but an intensified, reaction*, of such a sort that one is not justified in regarding the question as one of actual immunity. The death occurring after the second or third injection renders superfluous any theoretical discussion as to whether or not an immunity was developed.

In serum disease this question might in itself bear discussion, since the intensified and hastened reaction might here be an indication of immunity; but there is no *essential* difference in the action of serum and organ albumen, although the one leads merely to an accelerated and intensified reaction and the other to death.

It appears necessary, therefore, for other than systematic reasons, not to confine one's investigations to the subject of serum albumen, since the idea so obtained would necessarily be one-sided. By comparing the findings in injections of organic and bacterial albumen one's conceptions are broad-

ened. The experiments with serum disease are, therefore, only a part of all the experiments performed, and are, moreover, the tests made with the least active poison.

Organic albumen, in contrast with serum albumen, is also morphologically organized. Unlike serum albumen, which cludes microscopical demonstration after injection, organic albumen can be traced microscopically for a long time after the injection. We may, therefore, make the significant and interesting observation, with regard to bacterial and organic albumens, that after a repetition of the injection, the lysis of the cells and bacteria proceeds more and more rapidly, and that this accelerated lysis is directly and distinctly related to the 'accelerated and intensified' reaction. This relationship can be traced *directly*, whereas Pirquet, Schick, and other authors absolutely failed to determine any direct relationship between the precipitin formation which they assumed and serum disease.

As a matter of fact, however, these substances act as a highly important and valuable means of protection, but only in the presence of certain conditions. Wolff-Eisner (*Berl. Klin. Woch.*, 1907, No. 38, p. 18) sums up this view as follows:*

'In immune animals, therefore, the bacteria are destroyed so quickly that the amount of the fatal dose is hardly ever approached.'

Significance of Accelerated Bacteriolysis for combating Infectious Diseases.—'The acceleration of bacteriolysis is so great that under these circumstances hypersensitiveness to endotoxins is not especially concerned in the matter. Hypersensitiveness is really the greatest paradox in immunity. Instead of the immunity one would expect after the injection of albuminous substances, a hypersensitiveness occurs. And yet hypersensitiveness is not altogether purposeless: for, apart from some relatively rare cases, large amounts of heterogeneous albumen are introduced into the system only in experiments; more often bacteria constitute the heterogeneous albumen, and the very hypersensitiveness of the organ to the albuminous substance in question seems to concentrate the bacteriolytic forces of the body against

* *Cf.* also remarks on p. 66 of this work.

the invading bacteria. From this hypothesis it is evident how higher organisms have been able to survive the struggle for existence, in spite of a hypersensitiveness apparently so injurious.'

Wolff-Eisner says, further, in his work 'Frühdiagnose und Tuberkuloseimmunität,' 1909, p. 311: 'The favourable effects of tuberculin hypersensitiveness are in tuberculosis, as in other bacterial infections, dependent upon hypersensitiveness, and consist in preventing the development of secondary foci. The dangers to the patient in hypersensitiveness must by no means be overlooked. The word "teleology" is always to be regarded with suspicion in pathology.'

Pirquet in 1905 ('Serumkrankheit,' p. 134) says, regarding this point: 'In infectious diseases we see distinctly the advantage—the teleologic significance, so to speak—of the hastened reaction. The earlier the reaction of the organism occurs, the less time the invading substances have to proliferate.'

Thus far the views of Pirquet coincide with those of the author; but Pirquet considered it possible that a toxic action might be brought about solely through an interaction of antigen and antibody, while Wolff-Eisner considers the liberation of toxins by lysis, with the aid of complement, as indispensable. This, as just mentioned, is the only distinction between our independent and almost simultaneous observations.

As has been mentioned, Pirquet has been compelled by his observations on revaccination to acknowledge the significance of lysis.*

* Pirquet ('Revaccination,' 1907, p. 188) says: 'The first antibodies that appear are those directed against the enveloping substance of the bacteria; only later do those antibodies appear which, being called forth by the action of the cell-toxin, are directed against it.' P. 189: 'These conclusions are new. They culminate in the view that the inflammatory symptoms are produced with the assistance of antibodies.'

That this view of the significance of lysis in the phenomena of infections *was not original with Pirquet* is evident from the foregoing (*cf.* p. 66). Moreover, Pirquet does not unreservedly adopt my view-point, but advances a dualistic theory for the phenomena of revaccination, differentiating (1) lysins directed against the enveloping substance, and (2) subsequent antitoxins. There is also no reason for surrendering the author's view, embracing almost all infections, on revaccination.

Pirquet, however, assumes a very complicated structure of antibodies. Besides the antibodies dissolving the capsule (lysins), he assumes antitoxins acting upon the contents From the varying proportions of these lysins to antitoxins the manifold forms of phenomena are said to be deduced. For this, however, the lysins fully suffice, and it is much simpler to explain the reactions by one group of demonstrable reactive substances than to introduce new and entirely hypothetical substances into the question.

Pirquet ascribes exanthema, particularly that of smallpox, to agglutination, although Kraus and Sternberg have proved that no precipitation takes place in the body-juices, although Pfeiffer and his school have always pointed out that a bacteriolytic serum with a high agglutination titer does not exert an agglutinating action upon typhoid bacilli in animal experiments, and although Pirquet has himself observed that in typhoid fever there is no general exanthema, in spite of the known power of agglutination, but merely roseola on the body. However, the freeing by lysis of heterogeneous albuminous substances is entirely sufficient to explain the occurrence of exanthema analogous to urticaria, which, as Pirquet himself had proved in serum disease, is in no way related to precipitins.

The comprehensive works of Friedberger and his school show the same views in all essential points as are here advanced, particularly that the mechanism of hypersensitiveness is analogous to hæmolysis, since the co-operation of antigen and complement is necessary.

The fact has recently been established in Friedberger's investigations that it is possible to liberate the substances bringing about hypersensitive symptoms through bringing together in a reagent glass antigens, antibodies and complement, and to establish the quantitative factors.

Whether the liberation of this anaphylactic toxin *in vitro* will prove constant will have to be proved by further experimentation, since hypersensitiveness depends not only on the

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After this evidence the author feels himself obliged—with all due deference to the merits of Pirquet—to take exception to his intentional fiction that the credit is due to him for the explanation of these conditions.

poison, but also on the varying action of the poison on the organism, which important factor seems to have been overlooked by Friedberger. It must also remain for further investigation to find whether the Friedberger claim, that the poison liberated from albuminous substances is a simple anaphylactic toxin, is justifiable.

The proofs presented by Friedberger—viz., that various forms of fever can be produced with albumen anaphylactic toxin—are inadequate. Furthermore, he believes that symptoms of hypersensitiveness are induced by insufficient splitting up of albuminous substances. He therefore believes, contrary to the author, in quantitative differences between enteral and parenteral ingestion of albumen.

History of Hypersensitiveness.

After the outline given above of the nature of hypersensitiveness, and after an analysis of its clinical forms, we may briefly discuss its history.

Behring was the first to describe the phenomena of hypersensitiveness and to ascribe it to toxins. Richet next showed that in one specific instance—that of the poison of Medusa—a hypersensitiveness took place instead of the insensitiveness one would expect. This case report, however, does not justify us in regarding Richet as the inaugurator of the great theory of hypersensitiveness, any more than would the fact that he gave to hypersensitiveness the term 'anaphylaxis,' which term conveys no idea of the nature of the subject, and therefore has not been used in this chapter. Pirquet, in his articles on serum disease, revaccination, and cutaneous reaction, was the first to give a concise clinical description and theory of hypersensitiveness which would bear discussion. Wolff-Eisner at the same time published his fundamental law of immunity—viz., that the injection of all heterogeneous albumens produces, not insensitiveness, but hypersensitiveness. He showed by experiment how to recognize the fatal syndrome of hypersensitiveness, and gave in his works on hay-fever, urticaria, tuberculous infection, and

tuberculin reaction the first data of spontaneous diseases of hypersensitiveness.

The same version of the development of the subject is given by Kraus and Dörr, Römer and Much. The last-named authors object to the term 'anaphylaxis,' which is really the only claim Richet has to offer as the discoverer of hypersensitiveness. The honour belongs either to Behring or to those authors who first reported cases. Richet worked with a primarily toxic albumen, a partial toxin, whereas the nature of hypersensitiveness is such that a repetition of the injection causes a severe toxic action. Furthermore, his experiments were made with a substance void of any practical significance. However, this work is more than a mere case report, since he determined the invariability of the occurrence of hypersensitiveness. But the discovery of hypersensitiveness as a law governing all biology dates from that moment when it was determined that the injection of any heterogeneous albumen (organ serum or bacterial albumen) produced, not an immunity in the usual sense of the term, but a uniform hypersensitiveness.

We may question, as does Dörr, whether Behring's hypersensitiveness to toxins belongs to the category of hypersensitiveness to heterogeneous albumen. A definite decision cannot now be given. However, taking it all in all, it is probable that in hypersensitiveness to toxins we also have to deal with an analogous process. It appears as if the toxins, which otherwise deflect from the law outlined above, recall their former albuminous character. In the opinion of the author, Nicolle's theory promises light on the subject: If coagulins are preponderant, antitoxins are formed; if lytic components predominate, hypersensitiveness results.

This theory seems to offer a working hypothesis, by means of which it may be possible to understand the interaction between the formation of anti-endotoxin and hypersensitiveness after the injection of albuminous substances, particularly those of a bacterial nature.

CHAPTER VI

Precipitins—Precipitin production—Precipitin experiment—Clinical significance—Agglutinins.

Precipitins.

THE property of precipitins to yield a precipitate, especially with the particular serum with which they were developed, has long been of practical importance. It may even be said that the application of precipitins marked the beginning of sero-diagnostic methods.

The method is based upon the fact that every species of animal possesses its own particular kind of serum, acting differently from that of all other species. The ordinary practitioner has little to do with precipitins, since they are used only in legal cases; yet since these methods are important from a criminological point of view, and the decision of the court often depends upon their outcome, every physician ought to know their nature and principles. Moreover, a knowledge of precipitins is becoming very important in infant feeding, and in understanding the processes of intestinal digestion as well.

Before the precipitin reaction was known, it was as a rule difficult to determine the presence of blood in criminal cases. Everyone remembers producing Teichmann's and other blood crystals in his student days. Yet even after these were obtained nothing was proved further than that we were dealing with blood; there was no way to determine from what species of animal the blood was derived. For this reason these methods were of minimum forensic value.

Forensic Significance of Precipitins.—The precipitin reaction, making possible an exact determination of the species of animal from which the blood was derived, completely revolutionized this state of affairs. It is of the greatest importance from a legal standpoint that the precipitin reaction is in nowise affected, even though the blood has been dry for some time. Scientists have even succeeded in obtaining a positive precipitin reaction in mummies.

The test is applied as follows: The piece of goods containing the spot suspiciously resembling blood is softened in normal salt solution, and the precipitin in various degrees of dilution is added to the filtrated solution. If a precipitation now takes place with human precipitin,* it is safe to conclude that the spot contains human blood.

Nomenclature.—We would in this case designate—

1. The solution, prepared from the blood-spot as precipitable substance.
2. The serum applied for precipitation as precipitin.
3. The substance obtained by the action of the precipitin upon the precipitable substance as precipitate.

Since a positive reaction may be of the greatest moment, it is hardly necessary to emphasize the fact that the investigator (a medico-legal expert) must use every precaution and make numerous controls. For instance, a cloudiness due to the development of bacteria in the tubes must not be mistaken for a precipitation. It must also be remembered that group reactions occur. A precipitin obtained by injecting human serum into a rabbit would give a precipitation with the blood of a monkey. To be sure, these so-called 'partial precipitins' may be removed by allowing the precipitin to act upon monkey serum, and, after precipitation, separating the precipitate by filtration or centrifugalization. The precipitate then consists of monkey serum and the partial

* The precipitation is recognized by the cloudiness occurring when the two clear liquids (precipitable substance and precipitin) are mixed. The lowest degree of precipitation consists in a milky opacity; medium and higher degrees in a precipitation of flakes, which gradually form a sediment on the bottom of the glass.

Precipitation is best observed with a magnifying-glass

precipitin which the human blood possesses for monkey serum. The remainder of the precipitin is now tested with monkey and human serum, and shows, if the serum used was obtained by the injection of human serum into an animal, and if all partial precipitins have been removed, a precipitation with human serum, but no longer with that of a monkey.

Weichardt has attempted in this way to determine special race, and even individual, precipitins. What his methods gain in refinement they lose in clearness, and these serological refinements cannot be recommended for legal purposes.

In using precipitins, one must always remember that the precipitate dissolves in an excess of precipitin or precipitable substance. The precipitable substance must therefore always be applied in dilution.

Preparation of Precipitins.

The preparation of precipitating serum is very simple. An animal, let us say a rabbit, is injected several times (subcutaneously with 20, 10 and 5 c.c., intraperitoneally with 10, 8 and 5 c.c., or intravenously with 5, 3 and 2 c.c.—hence in a descending scale) with a certain serum. After, perhaps, the third injection, the titer of the resulting precipitin serum is tested.

The Precipitin Test.

This is made in various ways, according to whether we wish to demonstrate precipitable substance or to determine how much precipitin a serum contains.

The first case is the more important from a practical viewpoint. We have a precipitating serum with a titer of 1,000—*i.e.*, 1 c c. of the serum still precipitates human serum in a solution of 1 : 1,000, diluted about six times. One fourth of a c.c. of precipitin is now added* to various dilutions of the liquid to be tested, and the occurrence of a precipitation is determined after an incubation of from half an hour to two hours at 37° C.

On the other hand, in titrating the precipitin, a precipitable substance of known origin, such as human serum, is diluted five to ten times, and to each c.c. is added $\frac{1}{4}$ c.c. of dilutions of the precipitin serum to be tested. The dilutions in which precipitation still occurs indicate the precipitin titer of the serum.

* Partly undiluted, partly in a solution of 1 : 10.

PROOF OF PRECIPITABLE SUBSTANCE. (RECORD OF EXPERIMENT.)

Liquid to be tested for Precipitable Substance.	+ Precipitin (Titer 1,000, 1 c.c. of a 1 : 10 Dilution).	Result after Incubation of One Hour at 37° C.: Precipitation.
Dilution { 1 : 5	1 c.c. 1 : 10	+ + + (strong precipitation)
1 : 10		+ + + ,, ,,
1 : 50		+ + + ,, ,,
1 : 100		+ + ,, ,,
1 : 500		+ (precipitation)
1 : 1,000 }		— (no precipitation)

Result: The precipitable substance is precipitated by the serum up to a dilution of 1 : 1,000.

The Clinical Significance of Precipitins.

Aside from their legal significance, precipitins are daily becoming more important in the physiology and pathology of digestion, particularly in gastro-intestinal affections of infants.

We now know that the subcutaneous, intraperitoneal or intravenous introduction of heterogeneous albumen elicits the formation of precipitins. On the other hand, it may easily be determined that no precipitins are contained in our own serum, even after ingesting milk, raw eggs, or raw meat. Thus ingestion *per os* (enterical) of albumen differs from the methods above mentioned (subcutaneous, etc.).

Process of Normal Digestion.

The intestinal juices (pepsin, trypsin, and enterokinase) act upon the albumen introduced *per os*. They split up the heterogeneous albumen in such a way as to deprive it of its specificity. Not until then is it reabsorbed, and the molecules of broken-down albumen now constitute the material from which the organism builds up its own native albumen by synthetic processes, the details of which are not yet known.

These conclusions are drawn from the following experiments and statements:

1. The precipitable substance is so altered by the digestion with pepsin and trypsin that it no longer gives a precipitate with precipitin (Michaelis).
2. Albumen, digested with pepsin or trypsin, loses its

property of producing precipitins in animals when injected enterically (Michaelis).

3. That analogous processes must be assumed in the animal body follows from the fact that in the serum of normal man, *e.g.*, even after continued ingestion of raw eggs, neither the white of the eggs (precipitable substance) nor precipitins against the white of egg are found in the serum.

This holds good only in normal conditions. Under pathologic conditions, especially when, by way of experiment, greater amounts of albumen have been introduced into the intestines than the animal would have ingested under normal conditions—*e.g.*, overfeeding of rats with blood, and the like—unaltered heterogeneous albumen passes over into the circulation, and produces the corresponding reactive substances (antibodies).

Regulations for Preventing the Reabsorption of Heterogeneous Albumen from the Intestinal Tract.—Under normal conditions extremely delicate regulative mechanisms prevent the unaltered heterogeneous albumen from passing through the intestinal wall into the body juices. The following illustration will show how delicate these mechanisms are. A reabsorption by the intestine of heterogeneous albumen, not sufficiently split up, is possible only when this heterogeneous albumen is in an absorbable—*i.e.*, liquid—form. Cow's milk is a heterogeneous albumen which is ingested in large quantities in liquid form. However, the milk is caseated almost immediately by the lab ferments of the stomach—*i.e.*, it is changed to a solid form. As the intestinal function generally consists in transforming the substances ingested into a liquid and absorbable form, this reverse process until recently seemed inexplicable. It is now apparent that it is for the purpose of obviating the reabsorption of liquid heterogeneous milk. Under the influence of the intestinal ferments the milk is again liquefied. Thus there is a possibility of reabsorption only in case the heterogeneous milk has been deprived of its specificity, or if the production of lab is insufficient.

Differences between Cow's Milk and that of Mothers.—These statements have a fundamental significance as regards

the question of artificial infant-feeding. It was believed until only a short time ago that the difference between cow's milk and that of mothers consisted only in differences of the content of albumen, fat, and sugar; and physicians sought to make cow's milk like that of the mother by correcting these differences. The chief difference, and one that cannot be eliminated by adding water and milk-sugar, lies in the character of the albumen of the two kinds of milk.

It is a well-known fact that the mortality is greater among children brought up on cow's milk than among those that are breast-fed. Even after bacterial agents not present in mother's milk are eliminated, the mortality of bottle-fed infants is still in excess, especially from gastro-intestinal diseases.

The above-described measures taken by the body against the reabsorption of heterogeneous albumen protect the infant against the entrance of such albumen while insufficiently split up. Only in exceptional cases have precipitable substances (precipitin) been determined in the blood of infants suffering from atrophy. How, then, is the excess of mortality in bottle-fed over that of breast-fed infants to be explained?

The infants that are breast-fed can reabsorb the native milk unaltered. The intestinal glands need not functionate. This premature functioning of the infantile intestinal glands, the premature secretion of the intestinal ferments, necessitates a considerable increase of work for the bottle-fed baby in contrast with what is required by the breast-fed infant (Wassermann). As additional work necessitates additional food, more of the heterogeneous albumen must be added, and thus, in a way, a vicious circle is established.*

Injurious Effects of Cow's Milk.—The effects of this early functioning of the intestinal glands vary with different

* The views of Escherich and Pfaundler, that in the milk of the same animal species, aside from the nutritional substances, complements necessary to assimilation are also present, depends upon Ehrlich's original theory of the analogy between the processes of immunization and the physiology of nutrition. It has already been mentioned that this is not quite consistent, and the author, with Nöggerath (*Deutsch. Med. Woch.*, 1909, No. 43), regards this view as incorrect, the more so since the absorption of antitoxin through the intestinal wall proves nothing as regards the similar disposition of complements.

children. With some no ill effects are observed, the child develops normally; with others a certain instability of the digestive processes results. The overworked intestinal tract is a *locus minoris resistentiæ*, and from time to time intestinal catarrhs develop, necessitating a temporary suspension of the milk. In other infants an atrophy develops, leading to death if the diet cannot be changed to the milk of mother or wet-nurse.

Theory and experience lead to the same conclusion—viz., whenever it is possible the nutrition of the infant should be carried out with the milk of the mother or wet-nurse. This has always been admitted; however, the credit for having explained the reasons for feeding with mother's milk is due to investigors in the field of immunity. The credit will be due to immunity if, in the future, the belief does not again become prevalent that it is modern and scientific to rear infants by means of the bottle.

The majority of pediatrists believe that the significance of heterogeneous albumen is not worthy of recognition, since its chief value depends on fats and serum lactis concentration. The results produced with Finkelstein's albumen-milk have supported this view. Yet these results are satisfactorily explained, when we take into consideration the fact that secondary injuries to the intestine are avoided by preventing the carbohydrate fermentation processes.

Here, as so often happens, narrowness led to mistaken views. During the first period, only the bacteria (Soxhlet) were taken into consideration; during the second, only the heterogeneous albumen. At present only chemical and chemo-physical factors are usually regarded, whereas all three factors work together for the same end.

Recent investigations of Abderhalden (*Med. Klinik*, 1910) throw a new light on this process, and bring out new evidence in support of the lytic theory of the action of heterogeneous albuminous substances. Abderhalden caused ferments to act on chemically known substances similar to albuminous substances, so-called polypeptids. He was able to demonstrate the various stages of the splitting-up process by an alteration of the optical rotatory powers. In parenteral ingestion of albumen, the body forms ferments resembling in their action intestinal ferments. He cites the following experiments: Normal horse serum does not split up certain poly-

pepteids, but the serum of a horse which has been previously treated with heterogeneous albumen will do so.

Experiments show that the body, upon parenteral introduction of albumen, forms substances similar to digestive ferments which effect a splitting up, or, as the author has expressed it, from a morphological-bacteriologic point of view, a lysis. The process is similar to digestion. That it is not exactly analogous, and that a complete splitting-up of the heterogeneous albuminous substances is therefore not successful, shows (contrary to Abderhalden's opinion) a sensitiveness toward parenteral ingestion of albuminous substances.

The difference between native and foreign albumen is the chief problem of infant pathology. In the adult the digestive organs are better fitted to fulfil their function. However, a great many intestinal affections will doubtless be found attributable to heterogeneous albumen, which is difficult to break down. The same conditions are present in the infant also, not, as a rule, in the assimilation of heterogeneous albumen into the circulation, but in the form of an alteration of the digestive organs.

We know from clinical experience that the albumen of crabs and lobsters belongs to the albumens that are difficult to assimilate, and the splitting up of which imposes a great deal of labour upon the intestinal glands. A mere indigestibility would not cause as frequent gastro-intestinal disorders, since cellulose and similar indigestible 'ballast' does not usually affect the gastro-intestinal canal.

The author was the first to point out that a number of so-called idiosyncrasies, such as the occurrence of urticaria, particularly after the ingestion of lobster or strawberries, was very likely attributable to the reabsorption of small quantities of heterogeneous albumen which has not been split up because of an insufficiency of the digestive ferment. (*Cf.* chapter on Hypersensitiveness, Urticaria, p. 60.)

Experiments of French authors have also proved that even in adults nutrition with heterogeneous albumen implies an increase of activity, in contrast to nutrition with native albumen. These experiments show that for the maintenance of bodyweight, and for increasing the weight of the animal, greater amounts of heterogeneous albumen are necessary

than when nourishment consists of native albumen (*e.g.*, meat of the same species).

Agglutinins.

A twofold value may be ascribed to agglutinins:

1. Diagnostic, in infectious diseases, inasmuch as agglutinins may be demonstrated in the serum of the patient. For this a reliable test culture of the bacteria in question is required.
2. In identifying unknown or uncertain bacteria. A serum containing agglutinins is necessary for this purpose.

Agglutinins (apart from the bacteriolysins to be spoken of later) are formed when living or dead bacteria are injected into an animal, and sufficient time elapses for their formation before the animal succumbs from toxæmia. The formation of agglutinins is specific. For example, after the injection of typhoid bacilli only agglutinins for typhoid bacilli are formed. This is why we may conclude from the presence of agglutinins in the serum that the exciter of the infection which has given rise to their formation is, or has been, present in the animal's body.

The agglutination test, the clinical application of which was one of the first discoveries in immunity investigation, is of great clinical significance. The diagnosis of typhoid fever by the agglutination test is usually, though incorrectly, known as 'Widal's' phenomenon. The term 'Gruber-Widal' reaction is more nearly correct, but we must still bear in mind that it was only through the researches of Richard Pfeiffer, Kolle, and others, that Gruber and Widal were enabled to apply the agglutination test clinically in typhoid fever.

The Agglutination Test in the Clinic—Widal's Test.—The clinical application of the agglutination test marks an entirely new epoch in diagnosis. Only through this test and other methods have we learned that typhoid fever is far more common than was formerly supposed. Whereas this disease was formerly recognized only by a clinical symptom-complex regarded as characteristic, we now know that there are numerous atypical cases of typhoid differing in no way from

cases of sepsis so far as the course of the fever and other symptoms are concerned. A diagnosis of these cases cannot be made by mere clinical observation.

A diagnosis of typhoid fever on the ground of a positive Widal reaction is carried out as follows: The serum is tested for its content of agglutinin for typhoid bacilli. Blood is taken from the patient, and the serum allowed to separate. It is then tested in different dilutions with typhoid bacilli, by adding an equal amount of typhoid bacilli to 1 c.c. of the serum dilution—*e.g.*, a platinum loop of a twenty-hour agar culture—or by adding the various serum dilutions to 1 c.c. of a twenty-hour bouillon culture. The tubes inoculated are incubated at 37° C. The agglutinin content (the titer) is determined by observing up to what dilution, in half an hour to one hour, an agglutination appears.

The method in this agglutination test is as follows:

SERUM TEST FOR CONTENT OF AGGLUTININ (WIDAL).

Patients' Serum.		Result.		
		At Once.	After Half an Hour at 37° C.	After One Hour at 37° C.
1 c.c. of 1 : 10 dilution	To each tube add one loop of a twenty-hour agar culture of known typhoid bacilli, and mix thoroughly so that a fine emulsion results	+ +	+ + +	+ + +
,, ,, 1 : 20 ,,		+	+ +	+ + +
,, ,, 1 : 50 ,,		—	+ +	+ +
,, ,, 1 : 100 ,,		—	+	+
,, ,, 1 : 200 ,,		—	—	—
Control tube: 1 c.c. normal salt solution		—	—	—

+ + + = strong agglutination. | + = weak agglutination.
+ + = distinct agglutination. | — = agglutination absent.

What is Agglutination?

The nature of agglutination is not yet fully clear. It is supposed that under the influence of agglutinins the delicate ciliary appendices of the motile bacteria become swollen, adhere to one another, and in this way form accumulations or clumps. The phenomena observed in the agglutination test fully support this conception. If the bacteria in the serum dilution have been well macerated, the solution appears uniformly opaque when observed with a magnifying-

glass.* If agglutination occurs from the action of the agglutinins present in the serum, we may recognize, either macroscopically or with a magnifying-glass, small clumps, which give the uniformly opaque emulsion a granular appearance. If the agglutinin content of the serum dilution is a multiple of the necessary quantity, agglutination may occur instantaneously at room temperatures. If these tubes are kept for some time in the incubator, the clumps become gradually larger, sink to the bottom by their own weight, and the supernatant liquid becomes clear. If the mixture is shaken no emulsion results, but the clumpy formation persists. This macroscopic method of determining the agglutinin content of a serum is at present the most generally employed, yet it is occasionally replaced by the microscopic method, according to which, one drop of the serum dilution containing the bacteria in question is examined with proper blending under a low magnification, and the presence of clumpy masses of bacteria is determined. (Clumps which can be found only with the immersion lens are not, as a rule, regarded as having any significance in the microscopical determination of agglutinin content.) The first appearance by which one may determine the alteration of motile typhoid bacteria upon which agglutinins have acted is the absolute loss of previous motility, due to the hypothetic disturbance of the ciliary apparatus.†

Nature of Agglutinins.—The question of the nature of agglutinins is related to the statement above-mentioned, that after the introduction of bacteria agglutinins and bacteriolysins are always formed simultaneously. Bacteriolysins possess a complicated amboceptor structure; *i.e.*, they can act only when the complement has been fixed to them. It has been suggested that we assume that agglutinins associated with bacteriolysins are identical with the latter; and that in the presence of complement the bacteriolytic, and in the absence of complement the agglutinating, action is exerted upon the bacteria. This explanation seemed to be con-

* Under an immersion lens the bacteria must lie separated.

† The vitality and the capacity of typhoid and other bacilli for causing infection is not impaired by agglutination.

firmed by the fact that an agglutination frequently precedes bacteriolysis as well as hæmolysis. However, later discoveries have been made which indicate that agglutinins and bacteriolysins are indeed different bodies.*

Many difficulties exist in the clinical application of agglutination findings, which the clinician must know if he wishes to avoid diagnostic errors himself.

Clinical and Diagnostic Significance of Positive Agglutination Findings.—For many years after an infection agglutinins remain in the serum, a sign as it were of a preceding infection. Thus, agglutinins do not always indicate an existing infectious disease, but may show a previous infection as well. A careful clinical history does not always clear up the matter, for there are infectious diseases the nature of which may not have been recognized; and some may be so mild that the individual affected may not know of their existence. This does not apply alone to that class of individuals who, because of deficient intelligence, pay too little attention to their physical condition.

On the other hand, the absence of agglutinins may mislead diagnosticians unless it is borne in mind that the formation of agglutinins and their appearance in the serum requires time. A negative agglutination finding at the beginning of a disease proves nothing, and the test should be repeated after a short time.

Positive Typhoid Agglutination in Diseases of the Gall-Passages.—A theoretical difficulty in the way of the diagnostic application of agglutination findings lies in the fact that the presence of typhoid bacilli have been observed in a number of other diseases, particularly affections of the bile-ducts. This peculiar phenomenon can only be explained by the so-called 'co-agglutination,' according to which it is understood that other bacteria more or less related are co-agglutinated by an agglutinin. The question is one of a so-called 'group reaction,' and the phenomenon is therefore called 'group agglutination' or 'co-agglutination.'

* The fact should here be mentioned that agglutinins and bacteriolysins differ in the degree of their preservability; furthermore, that bacteriolysis may occur without a previous agglutination; and that, finally, the predominance of agglutinin or bacteriolysin formation depends largely upon the technic of injecting bacteria.

The question of the specificity of agglutination is not as a rule complicated by group agglutination. Group agglutination is explained by assuming a receptor apparatus at least partially common to the related bacteria.

Co-Agglutination — Castellani's Experiment. — If the limits of the capacity of a serum for agglutinating two different bacteria are very far apart—*e.g.*, if the titer of agglutination for typhoid bacilli is 1 : 1,000, and for colon bacilli 1 : 100—we assume a mere co-agglutination. If the titer for both kinds of bacteria is very similar, we attempt to differentiate the organisms by means of Castellani's experiment. Two specimens of the serum are saturated with the bacteria in question, which, after being allowed to stand for twelve hours, are separated by centrifugalization. In the first specimen, only those bacteria for which the serum after this treatment has lost its capacity of agglutination are co-agglutinated.

By this method gonococci, for example, are separated from meningococci, although otherwise very closely related biologically, and are, for instance, agglutinated by the same serum in an equal dilution. The theory of a common receptor apparatus not only explains co-agglutination, but also the fact that in other diseases, especially those of the bile-passages, co-agglutinations are formed. We may assume in these affections that the bacteria related to typhoid bacilli, particularly *Bacterium coli*, produce agglutinins in the serum of the patient which co-agglutinate typhoid bacilli.

There is no relation whatever between the content of agglutinins of the serum and the severity of the affection, especially between the amount of agglutinins and the prognosis of the case. Contrary to other opinions, it should be kept in mind that cases with a high agglutinin content may die; while, on the other hand, cases with a low agglutinin content may recover.

Ficker's Test as a Substitute for that of Widal.—Despite the great clinical significance of the so-called 'Widal test,' it cannot be applied in general practice, but is confined to hospital work, for the reason that the test requires living typhoid bacteria. Because of the danger and inconvenience

of living typhoid cultures, the reaction can only be carried out in specially-equipped laboratories. Recently, however, the test has been modified by Bordet and Ficker, so that its use has become more general. Instead of living typhoid bacilli, emulsions of the specially-prepared dead bacilli are used. The reaction is determined by observing whether a clarification and sedimentation occur in the slightly cloudy liquid within twenty hours. We have to deal here, not so much with an agglutination as with a precipitation of the bacterial extract. This method is more convenient and safer than the Widal, and gives good results. The original Widal test, however, is vastly superior to the Bordet-Ficker modification test in point of exactness and reliability.

Determination of Identity of Cultures by Means of Agglutination.—The diagnostic use of agglutinins thus far described consists in allowing them to act upon known bacterial cultures. If an agglutination occurs, we conclude that reactive substances were present which had entered the serum of the patient under the action of the bacteria. However, a series of cultures is often obtained from patients, especially from the fæces in typhoid and dysentery, showing the morphological characteristics of typhoid, paratyphoid, dysentery, and other bacteria. The differentiation of such cultures is possible only with the aid of specific agglutination (or bacteriolysis) tests, since it has been proved beyond question that bacteria may exhibit all the characteristics of organisms of another kind—*e.g.*, of typhoid bacilli, while not being true typhoid bacilli.

On the other hand, in order to identify a culture by means of the agglutination test we must have agglutinating sera. An agglutinating typhoid serum is prepared by making a 'vaccine' of a known typhoid culture. This is done by emulsifying one or more loops of typhoid bacilli in 10 c.c. of physiologic salt solution, and killing the emulsion by heating for one hour to 60° C.; 1 to 2 c.c. of this emulsion is injected intravenously into rabbits or goats until the agglutination test exhibits a good titer.

Agglutination Test.—The agglutination test for the differentiation of bacterial cultures is carried out as follows:

			Result.	
Tubes containing each 1 c.c. of different dilutions of agglutinating serum of known titer—*e.g.*, 1:1,000	+	One loop of bacteria in question to each tube (thoroughly emulsify)	=	Immediately After half an hour at 37° C. After one hour at 37° C.	Positive or negative, according to the presence or absence of corresponding agglutinins

Application of Agglutination Method for Recognition of Non-Motile Bacteria (*e.g.*, **Tubercle Bacilli**).—The agglutination test has been tried for the identification of non-motile bacteria. Of course there is here no true adherence of cilia. However, since we have seen in Ficker's reagent that by combining the process of agglutination with that of precipitation dead typhoid bacilli may also be agglutinated, the same method may be applied to non-motile bacteria without any technical difficulty. Such agglutination tests have, indeed, been made for the identification of the majority of bacteria, but their diagnostic significance has in no case been as great as in typhoid fever, except in diseases of a typhoid character, such as dysentery.

A special interest is to be ascribed to the agglutination test in tuberculosis. The tendency of tubercle bacilli to grow matted together like threads made it extremely difficult to prepare an emulsion suitable for the agglutination test. Courmont and Arloing overcame this difficulty by their homogeneous tubercle bacillus culture. At present a well-homogenized preparation of tubercle bacilli, killed and ground in agate mortars, is put out by the 'Höchst Fabrik,' and used in the agglutination test for tuberculosis.

Agglutination tests in tuberculosis have a scientific, but as yet no diagnostic, value. Agglutinins are apparently formed in every tuberculous infection, and remain for a long time in the serum. At present there is no possibility of differentiating by their aid a healed from an active tuberculous process—a tuberculosis requiring treatment. Of late, agglutination experiments are again being made in tuberculosis, in the hope of regulating the action of the tuberculin-therapy by an ascending agglutination titer. Yet even here there is no apparent connection between the height of the agglutination titer and the course of the disease.

CHAPTER VII

Bacteriolysins—Opsonins—Hæmolysins.

Bacteriolysins.

As was explained in a previous chapter, two forms of immunity are to be differentiated—viz., antitoxic and antibacterial.

Antitoxic Immunity.—Antitoxic immunity is directed against the toxins produced by the bacteria, which are neutralized by antitoxins. The bacteria are thereby deprived of their aggressiveness in the true sense of the word. A further production of toxins would be of no advantage to the bacteria, since the antitoxin would again neutralize the newly-formed toxin, and only bring about an increase of the antitoxic power. From the failure of the antitoxic power to increase, we may conclude that the bacteria still present are merely saprophytes for the organism. However, it is important, from a clinical standpoint, to know that these bacteria may again develop an unimpaired virulence in another organism. In many cases the bacteria are sooner or later destroyed by the antibacterial power of the body. This will shortly be discussed in detail. However, since more attention has been given to bacilli-carriers in the suppression of infectious diseases, we know that it not infrequently happens that pathogenic bacteria remain as saprophytes in the individuals in question.

Antibacterial (Bacteriolytic) Immunity.—This form of immunity is directed against the bacteria themselves. It does not neutralize the bacterial toxins, but destroys the

bacteria. It is a difficult and fruitless task to try to decide which of the two forms better deserves the title 'immunity.' Antibacterial immunity destroys the bacteria, and, indeed, appears to strike at the root of the evil. It has, however, one characteristic which, under certain conditions may be of the gravest consequence: the bacteria are killed by being bacteriolyzed—*i.e.*, by being dissolved. In this process the heterogeneous albumen forming the bacterial body—the so-called endotoxins—is made absorbable, and the toxicity of the bacterial body substances may be considerable.

If at the time of bacteriolysis only a few bacteria are present, the small amount of toxin liberated is of no practical significance. If the bacteria have become numerous before their complete lysis was possible, this lysis may cause death. It is the immediate cause of death in many infectious diseases, and there is truth in the statement: 'No bacteriolysis, no death.'

Significance of Bacteriolytic Immunity.—The significance of bacteriolysis has only recently been recognized, for the reason that this apparent inconsistency offered some difficulty to the practitioner. Yet for this very reason it is absolutely necessary to understand the processes, for only thus is it possible to adapt indications and contra-indications of bactericidal sera to the individual case.

Great progress has been made during the past two years in the study of bacteriolytic processes. At present, however, there is a tendency not to consider bacteriolysis a process of immunization. This is a great mistake, for antibacterial (bacteriolytic) immunity protects us against diseases in innumerable instances, and does it so well that no discomfort betrays the fact that disease is threatening our bodies. Since the body is always exposed to bacteria, the antibacterial powers are obliged to resist continually the invasion of the various micro-organisms. If these bacteria are destroyed immediately upon their invasion, no symptoms of disease whatever occur, unless the invasion has been copious, or, as is more often the case, the invading bacteria have found time to proliferate before bacteriolysis begins.

Any power of Nature that serves humanity may also be

directed against it. Thus bacteriolysis, which so often decides the struggle with bacteria in favour of the higher organism, may also bring about disease and death; yet we misinterpret the fundamental facts if we try to separate bacteriolysis from immunization.

The significance of antibacterial is far greater than antitoxic immunity, for the simple reason that of the great number of pathogenic bacteria relatively few are producers of toxins; and, therefore, the formation of antitoxin has to do with only a few. On the other hand, the mechanism of antitoxic immunity is understood much more clearly than that of antibacterial immunity, in which investigators as a rule differentiate the bacteriolytic and phagocytic (opsonic) forms.

Should still Other Forms of Immunity (Opsonic, etc.) be differentiated from the Bacteriolytic Form?—The author holds to the opinion that the various forms of antibacterial immunity must be regarded as different manifestations of the same fundamental cause. It was long questioned whether any extracellular bacteriolysis (*i.e.*, a bacteriolysis outside the leucocytes) really took place at all. There is no longer any doubt of this. We know, however, that bacteria laden with bacteriolytic immune bodies are taken up more readily by the leucocytes—*i.e.*, are easily phagocytized, or 'sensibilized,' though the latter is a misleading term. If such sensibilized bacteria meet extracellular complement, they are bacteriolyzed; but should they first encounter a leucocyte, they are phagocytized. If leucocytes are present in great numbers, as in the so-called resistance experiment, it is impossible for an extracellular lysis to occur without an active phagocytosis taking place simultaneously.

These common examples show how intimately the two forms of antibacterial immunity are related, and how antibacterial immune bodies bring about either a bacteriolysis or a phagocytosis.

Direct and Indirect Methods for the Demonstration of Bacteriolysins.—The demonstration of bacteriolytic immune bodies (amboceptors*) may be direct or indirect. Direct

* Lytic immune bodies have a great many synonyms, which fact renders the subject more complicated. They are: bacteriolysins, hæmolysins, cytolysins,

proof is adduced by the plate method, or by observing bacteriolysis in the Pfeiffer experiment.

In demonstrating bacteriolysis by the plate method, bacteria are brought in contact with serum containing lytic amboceptors, complement is added, and the number of bacteria before and after the action of the serum is determined by the plate-counting method. Baumgarten and others protested vigorously against this method, pointing out that the destruction of bacteria might be due to other influences—viz., that bacteria are exposed to various osmotic influences, as the bacteria are inoculated successively from culture medium to serum, from serum to liquid, and then to solid culture media.

Pfeiffer's Bacteriolytic Experiment.—The Pfeiffer experiment is here of much significance. Bacteria, together with lytic immune serum, are injected into the peritoneal cavity of a guinea-pig. In unstained preparations we may satisfy ourselves at once that there really is an extracellular solution of bacteria—*i.e.*, bacteriolysis. The bacilli become swollen; they lose their rod shape, and become globular; their outlines become indistinct, and at last they disappear from view.

Besides this, the Pfeiffer experiment permits an exact determination of the amount of lytic immune bodies, a so-called titration of the serum.

Determination of the Amount of Lytic Immune Bodies of a Serum (Titration) by Means of the Pfeiffer Experiment.

For this one requires—

1. A serum for titration.
2. A fresh twenty-hour cholera culture of known virulence.
3. A number of guinea-pigs of 200 grammes.

The Pfeiffer Experiment.—In this case the titer of the serum is between 500 and 1,000, since, as the experiment shows, the animal in the second experiment survives and the

lytic immune bodies (or amboceptors), cytotoxins, sensibilisins, stimulins, and opsonins. We suggest, as the only terms to be used, lytic immune bodies, or amboceptors, or, more briefly, lysins.

THE PFEIFFER EXPERIMENT.

Guinea-Pigs of 200 Grammes.		Findings as indicated below—					
		At Once.	After Half an Hour.	After One Hour.	After Three to Five Hours.	After Twelve Hours.	After Twenty-four Hours.
Control animal	$\frac{1}{10}$ loop of cholera agar culture	Many bacteria	Many bacteria, some granules	Many bacteria, some granules	Many bacteria	—	+ (death) many bacteria
First experiment	1 loop of culture + $\frac{1}{100}$ c.c. immune serum	Many bacteria	Bacteria, Granules (equal quantities)	Granules only	Sterile	—	Lives
Second experiment	1 loop of culture + $\frac{1}{500}$ c.c. immune serum	Many bacteria	Bacteria, $\frac{3}{4}$ Granules, $\frac{1}{4}$	Bacteria, Granules (equal quantities)	Bacteria, $\frac{1}{4}$ Granules, $\frac{3}{4}$	Some bacteria, many granules	Lives
Third experiment	1 loop of culture + $\frac{1}{1000}$ c.c. immune serum	Many bacteria	Many bacteria	Many bacteria, some granules	—	Many bacteria, some granules	+ (dies)

animal in the third dies. The titer can be still more exactly determined by the same method.

In the Pfeiffer experiment it should be observed that ten times the fatal dose of the bacteria in question must be utilized, and, more important still, that the virulence of the culture used for the test must be of a certain degree, and that this virulence must be determined in every case by a control. So exact is the titer determination in this experiment that, unless the method is not strictly adhered to, the results are worthless.

The determination of bacteriolysins cannot be made as directly in all bacteria as in typhoid and cholera bacilli. The chief value of Pfeiffer's researches is that bacteriolysis has been shown to exist. All attempts to render the bacteriolysis of streptococci, staphylococci, and anthrax directly visible have proved failures.

Failure of the Pfeiffer Experiment for the Demonstration of Streptococci, etc.—There are two reasons for this. In the first place, the bacteria in the process of lysis lose their capacity for being stained to such an extent that even the granules of typhoid and cholera bacilli can be made visible for a short time only. Therefore, in the stained preparation only isolated granules can be demonstrated, while they may be shown in masses in unstained preparations. In the second place, the conditions are still more unfavourable with other bacteria. It is impossible to stain bacteria in the process of bacteriolysis, and, because of optical conditions, bacteriolysis is not demonstrable in the unstained fresh preparation.*

Indirect Demonstration of Bacteriolysins. — Since a direct demonstration of bacteriolysins by direct determination of bacteriolysis is unsuccessful in the great majority of bacteria, it is necessary to try indirectly to prove the exist-

* At the suggestion of the author, Markuse succeeded in rendering visible the process of lysis in anthrax and other bacteria by means of a special technique—viz., fixation of moist preparations in sublimate or osmium, staining with ferrous hæmatoxylin. Fritz Meyer and the author have also repeatedly believed that they saw extracellular lysis of streptococci under the action of antistreptococci serum. However, these findings were never as obvious as in typhoid or cholera.

ence of bacteriolytic substances in the serum. We have mentioned the fact that bacteria impregnated with bacteriolysins (typhoid bacteria also) are phagocytized more readily and in greater numbers. If such a stimulation of phagocytosis is observed, we may conclude, from the sensibilization of bacteria, that bacteriolytic immune bodies have been present in the serum.

We may also prove the presence of these substances by means of complement fixation, and comparative examinations of typhoid or cholera serum show that *by complement fixation substances are shown which are identical with lytic immune bodies* (*amboceptors*).

In the field of immunity there are many other facts indicative of the lytic nature of many of its phenomena. Wolff-Eisner has pointed this out both generally and specifically in the etiology of serum disease, hypersensitiveness to albumen, tuberculin reaction, and tuberculosis immunity.

*We therefore define bacteriolysins as reactive substances which form in the animal body as the result of the stimulus induced by the injection of bacteria or bacterial derivatives.** The function of bacteriolysins (in which they have the co-operation of extracellular complements) consists in dissolving bacteria, or in preparing the bacteria for ingestion and solution by leucocytes, probably with the aid of endocellular complements. The latter process seems predominant, especially in streptococci and in a number of other bacteria.

The mode of action of bacteriolysins is similar to that of hæmolysins. The author was able to show by means of nucleated erythrocytes how far this similarity extends, especially how the nuclei of the erythrocytes, which are harder to dissolve, are at first apparently destroyed only within the leucocytes, and how extracellular lysis also can be demonstrated with absolute certainty at the nuclei in Giemsa preparations (*Berlin. Klin. Woch.*, 1903, Nos. 17 and 20).

Bacteriolysins in serum may be demonstrated normally —*i.e.*, by means of the direct or indirect method. After the

* Cytolysins and albuminolysins are formed for cells and heterogeneous albumen respectively.

introduction of bacteria spontaneously (by disease) or experimentally, the amount of bacteriolysins contained in the serum increases considerably. By this deviation from the normal the bacteria are rapidly destroyed in the circulation, and thus a condition is produced which, in the great majority of cases, is properly designated immunity.

A high titer of bacteriolysins may sometimes be obtained by the injection of very small amounts of bacteria, even if killed.* Upon this fact are based the experiments in active immunization, especially in typhoid, cholera, and plague.

Bacteriolysins and Serum-Therapy.—The properties of bacteriolysins are applied in a practical way in the bactericidal form of sero-therapy. They are also used for the identification of cultures. For example, a typhoid-like bacillus, isolated from the stool, may be found to possess the cultural characteristics of typhoid bacillus. We are now able to determine by Pfeiffer's experiment, as well as by the agglutination test, whether or not the bacillus in question may be bacteriolyzed by a bacteriolytic serum at the titer value of the serum.

Identification of Bacteria by Bacteriolysins. — This method of identifying a culture is not often applied, since it is difficult and expensive, and especially since the isolated bacillus must have a certain virulence (see 'Pfeiffer's Experiment'). Moreover, this virulence is very often lacking, and has to be obtained by a series of animal transmissions.

In diseased individuals, particularly in typhoid patients, the content of bacteriolysins in the serum in the course of the disease has not yet been systematically determined. It has been mentioned that the content of agglutinins in the serum is in no direct relation to the course of the

* This, however, is conditional upon whether the bacteriolysins normally present are able to bacteriolyze the incorporated bacteria. This is the case with typhoid and cholera bacilli, but not with tubercle bacilli and their derivatives (tuberculin) in normal persons—*i.e.*, those entirely free from tuberculosis. This is why we do not obtain immunity by introducing derivatives of tubercle bacilli, particularly tuberculin, and why we do not, or only slowly and sparingly, obtain a formation of bacteriolytic amboceptors. These conditions have come to be of the most fundamental significance, since only in this way can the fact be explained that the conjunctival reaction in tuberculosis has a diagnostic significance, whereas the application of the conjunctival reaction in other infectious diseases, particularly in typhoid fever, although apparently so promising, has proved an utter failure.

disease. There is also no absolute relation between the content of bacteriolysins and the course of the disease, because, as we have seen, bacteriolysins are able to bring about a destruction of bacteria as well as the death of the animal. Whether the bacteriolysins cause recovery or death is largely dependent upon the second factor—viz., the bacteria—particularly their number. Nevertheless, we may expect to secure valuable evidence as to the nature of typhoid, of the processes in recovery from typhoid, and of typhoid relapses, by determining the bacteriolytic titer and observing the clinical phenomena.

Opsonins.

It is not by chance that we discuss opsonins immediately after bacteriolysins.

Definition.—The term implies that these substances render the bacteria palatable for leucocytes, without any inference as to whether the opsonins act upon the leucocytes or upon the bacteria. We remember, however, that bacteriolytic immune bodies have the same property as opsonins of 'sensibilizing bacteria,' or 'stimulating leucocytes for phagocytosis.' That both terms are used for the same process allows one to form an idea of the forces here becoming active.

Wright claims that there is a difference between opsonins and bacteriolysins. His arguments, however, are not sufficient to substantiate such a distinction,* and, moreover, a distinction of this sort would make the subject extremely complicated and difficult to understand.

Opsonic Index.—In opsonic experiments it is determined how much the phagocytic action of the leucocytes has been

* They consist in a difference in resistance to heat, as has been determined in a similar manner for normal and immune opsonins—*i.e.*, opsonins present in normal and immune persons. This fact is probably explained physicochemically by the various concentrations of opsonins and bacteriolysins in normal and immune serum (*cf. Münch. Med. Woch.*, 1901, No. 49, p. 2548: discussion at the third meeting of 'Freie Vereinigung für Mikrobiologie' on opsonins, bacteriolysins, bacteriotropin, and alexin). Dean has from the first advocated the view that opsonins are amboceptors, and Wright has even proved that diluted immune opsonin is thermolabile (*cf.* Sauerbeck, 'Krisis,' Leipzig, 1909, p. 37).

altered by a certain serum. However, there is no exact measure for this, and in every case the index is determined by comparing the opsonic value of the serum of a normal individual with that of the serum to be tested. If in 100 leucocytes 50 phagocytized bacteria are found in the serum tested and 25 in the normal serum, the relation is 50 : 25, and the opsonic index is therefore 2.*

Phagocytosis in opsonin experiments is very striking, and for this reason the impression has become general that the theory of opsonins would, if confirmed, mean the triumph of Metchnikoff's phagocytic theory. This view, however, is very superficial, and that the reverse is true is proved by opsonic experiments. The same leucocytes show a varying power of phagocytosis, under the influence of different sera. On the other hand, Wolfsohn and Wolff-Eisner have recently proved that leucocytes are also not wholly indifferent factors in phagocytosis (see ' Frühdiagnose und Tuberkulose Immunität ').

Opsonins and Bacteriolysins.—An idea of the content of opsonins in the various sera may be obtained by means of the method presently to be described. Opsonins, to which various terms are applied, are apparently identical with bacteriolysins. The most advantageous features of the opsonin determination are that the examinations may be repeated as often as desired, and that an idea at least may be obtained of the quantity of substances in the serum exerting such an action upon bacteria, whereas in the Pfeiffer experiment a direct estimation of bacteriolysins is impossible.

* Only relative values are obtained by opsonin tests. This is due to the fact that the leucocytes and the density of the bacillary emulsion cannot be prepared uniformly from day to day. For this reason it is necessary to re-examine the sera of normal persons in each opsonic series with that of the sera to be tested, since a comparison with former tests is only possible when the phagocytic count is compared every time with that of the normal serum. The phagocytic count varies on different days. The relation between the serum of the patient and that of a normal individual, other conditions being equal, is always the same.

The relation of pathologic serum to normal serum is called the phagocytic index. If it is less than 1 or 0 8, we have to deal with a diminution ; and if it is greater than 1 or 1·2, we have to deal with an increase of the phagocytic power induced by the corresponding serum.

Technique.—Of great importance, especially in opsonin determination, is technique.

The following are required for the purpose of estimating the opsonin content of a serum:

1. A mixture of leucocytes from a healthy person.
2. Sera from various patients and from one or two healthy individuals for comparison.
3. A bacilli emulsion.

The principle of the opsonin determination is as follows: Equal amounts of leucocytes, serum, and bacilli, are brought in contact with one another, and from the differences which appear conclusions are drawn as to the various amounts of opsonins or bacteriolysins in the serum. Since the technique employed is always the same, the only variation being that the serum used is from different persons, the variations in results must be ascribed to the differences in the sera used in the test.

The principle of opsonin determination is simple enough. The difficulties are purely technical. They are, at any rate, sufficient to make opsonin determination perhaps the most difficult and uncertain of laboratory procedures.

The opsonin content in a patient's serum must at times be repeatedly determined, and it is therefore important in carrying out the technique that one use the smallest possible amount of blood-corpuscles and serum. With this in view, an extremely delicate and withal clever technique has been worked out, which, it must be admitted, makes possible relatively rapid work. Nevertheless, many technical difficulties arise from the use of minute quantities of material.

The necessary ingredients for an opsonin test are prepared as follows:

Mixture of Leucocytes.

Preparation.—A fresh solution of 0·5 gramme of sodium citrate is added to 33 c.c. of distilled water. A test-tube 7 centimetres in length is repeatedly washed and filled three-fourths full with this solution. After congesting a finger, or preferably the thumb, by applying a gauze bandage approximately 1 centimetre in width evenly from the root to

the tip, and cleansing the part with alcohol, a pen-nib, half of which has been broken off and the remainder sterilized by heating red hot, is thrust into the dorsum of the finger near the bed of the nail. The blood is then allowed to flow into the test-tube containing the citrate solution (1·5 per cent.); or the opening of the test-tube may be placed tightly against the wound, and the blood (1·4 to 1·2 c.c.) allowed to flow directly into it. The solution of sodium citrate, added merely to prevent coagulation, must now be removed. The blood, therefore, after centrifugalization, is washed twice with physiologic sodium chloride solution (0·8 per cent.), as follows: The supernatant sodium citrate solution is carefully drawn off by means of a pipette capped with a rubber nipple; an equal amount of the above-mentioned salt solution is now added, the mixture shaken and centrifugalized for from five to ten minutes. The salt solution is pipetted off; the tube is again filled and centrifugalized. After removal of the salt solution, the remaining blood mixture contains the leucocytes necessary for the opsonin determination.

Obtaining the Blood-Serum.

The finger is congested in the manner above described. The blood is collected in a specially formed tube by capillary attraction (Fig. 1). The more conical the curved arm of the tube, the better the aspiration will be. To form this curve, the tube (preferably of Jena glass) is heated in flame to the proper degree, then, outside of the flame, is drawn slowly out and bent. The tube, filed and broken off, presents the appearance indicated by the figure. This tube takes up the blood from the puncture both rapidly and well.

FIG. 1.—TUBE FOR OBTAINING SERUM.

When the blood has been aspirated, the distal portion of the tube is carefully heated, and the capillary end melted in a small Bunsen flame. In cooling, the air enclosed in the straight arm of the tube is contracted and a vacuum formed, which draws the blood from the curved into the straight arm. Thus, the blood is

removed from the wall of the glass, and a separation of the serum is facilitated, especially if the tube is allowed to lie undisturbed for some time.

The Bacilli Emulsion.

Preparation of Bacilli Emulsion.—A bacilli emulsion must be so prepared that an average of only one or two bacilli are found to one leucocyte, for only thus is an exact count possible. The bacilli, therefore, must not lie too close together, and accumulations must be avoided.

The bacteria are emulsified by vigorous grinding in a mortar, and the proper concentration obtained by dilution.*

We now need only glass pipettes of uniform width, with a lumen of perhaps 1¼ millimetres at the lower end. Narrower tubes are not practicable for these experiments, as they do not permit an easy expulsion of the liquid.

The procedure henceforth is comparatively simple. The tube is marked with a grease pencil, and, with the aid of the rubber nipple, equal amounts of (1) blood mixture, (2) the serum for examination, and (3) bacilli emulsion are aspirated. The different parts are separated by a bubble of air. After aspirating (1) and (2) (mixture of leucocytes and blood-serum), it is well to remove the blood or serum adhering to the pipette with blotting-paper, or with the finger. The three separate parts are now mixed on the slide by repeated aspiration and expulsion. The tube is then sealed by melting at the pointed end, and placed in the incubator for fifteen minutes at 37° C. The tube is then broken at the capillary end, the liquid is expelled and repeatedly mixed, and a smear is made.

FIG. 2.—PIPETTE FOR OPSONIN DETERMINATION.

1. Mixture of leucocytes.
2. Serum of patients.
3. Emulsion of bacilli.

* There is a special method for preparing the emulsion of tubercle bacilli. Dead tubercle bacilli are ground in an agate mortar with a few drops of a 1·5 per cent. salt solution for one and a half to two hours, the saline solution

Smearing the Preparation.—The preparation of the smears would be very simple if only leucocytes were used in the mixture. As may be inferred from the description of the technique, a leucocyte mixture alone is not used, but a solution of blood which contains leucocytes only in relatively small numbers—an average of 1 leucocyte to 500 erythrocytes. In order now to count the phagocytic index without loss of time, it is necessary to concentrate the leucocytes at one point of the preparation. This is done as follows:

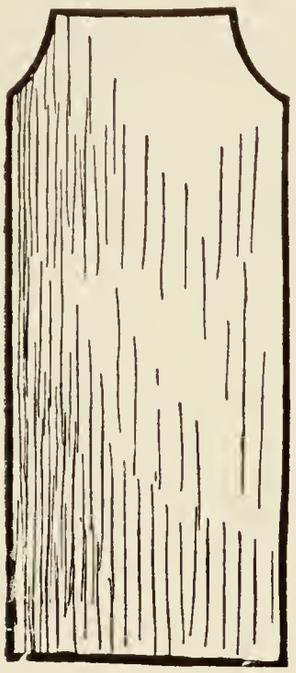

FIG. 3.—SPREADER FOR OPSONIN EXPERIMENT. Actual size.

A spreader is made by filing off and breaking the two edges of a glass slide having a very slight concavity at one end (Fig. 3). The blood is distributed by lightly drawing the spreader over the drop and smearing it smoothly, being careful to keep the drop under the middle of the spreader. The blood should be smeared on the convex surface of the object-glass. This surface is determined by spinning the slide on a plane surface, as a plate of glass, noting upon which side the slide spins. When the blood is spread in the manner above described, the lighter leucocytes are carried to the end of the slide, and in good preparations they are found lying in a row. To obtain a *good* smear, a good spreader is indispensable, and he who is fortunate enough to own such a one cannot be too careful in its preservation. It should also be mentioned that object-slides must be roughened with the finest French emery-paper before preparing the smear.

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being added drop by drop. A portion of the original solution above described is vigorously shaken, after which three parts of the 1·5 per cent. salt solution are added. This is thoroughly mixed by means of a pipette, by repeated aspiration and expulsion, and is then slowly centrifugalized for two minutes. After this the supernatant emulsion should be very slightly cloudy, and, when held against a black background, should present about the same degree of concentration or opacity as Ficker's typhoid diagnosticum.

Staining of the Preparations.

The technique for staining these smears differs somewhat from that ordinarily practised. The following directions must be strictly observed in order to obtain the best results:

1. The air-dried preparations are fixed in concentrated sublimate solution from three to ten minutes. (This concentrated solution is prepared by adding sublimate in excess to a 0·5 per cent. salt solution, and heating to the boiling-point. After cooling, it is filtered.)

2. After fixation, very careful irrigation in running water.

3. The staining is then accomplished with a solution of 1 per cent. thionin in 5 per cent. carbolic acid solution.*

Practical Significance of Opsonins.

According to Wright, a threefold significance is to be ascribed to opsonins—viz., diagnostic, prognostic, and therapeutic.

Diagnostic Significance of the Opsonin Determination.—The diagnostic significance depends upon the fact that the opsonic index is altered in certain infectious diseases—that is to say, it is either increased or diminished. Generally speaking, all values which exceed 0·8 to 1·2 are regarded as altered. From such increase or diminution of the opsonic index, as well as from great variations of the index, we may draw the conclusion that the individual is under the influence of the particular organism used in determining the index.

These conclusions are based upon empirical data, but they are explained theoretically by the observation that small amounts of bacterial substance raise the opsonic index and large amounts lower it. This explains the lowered or elevated

* The method of staining tubercle bacilli is different. The Ziehl solution, heated to the boiling-point, is applied. The solution is then washed off.

The specimen is immersed and gently shaken to and fro, not longer than six seconds, in 4 per cent. acetic acid (to decolorize the erythrocytes).

Decolorize carefully with 2 per cent. sulphuric acid. The preparation must have a reddish tint in water. Wash.

Counterstain for ten to twenty minutes in filtrated Löffler's methylene blue. Wash. Control intensity of staining with low power of the microscope.

index in infectious diseases, and also the fact that in certain infections the opsonic index is frequently subject to great variations.*

Prognostic Significance of Opsonin Determination.—Nothing definite can as yet be said of the prognostic significance of the opsonic index. We must not forget that the opsonins, or bacteriolysins, are only one of the factors determining the fate of the animal, and that death may occur in spite of the high content of bacteriolysins. Nevertheless, other things being equal, a low opsonic index must be regarded as prognostically unfavourable, if it is the result of an extensive reabsorption of bacterial substances or toxins. However, this must be proved in each instance.†

A high opsonic index has a certain favourable prognostic significance; nevertheless, it must always be remembered that opsonins, or bacteriolysins, are only one of the factors governing the fate of the infected individual.

Therapeutic Significance of the Opsonic Index.—The therapeutic indications afforded by a study of opsonins are of much practical interest. To be sure, the determination of the opsonic index will have little influence on our method of treatment of acute infectious diseases. There is no known

* Why the same infection causes in the one case a rise and in the other case a decrease of the opsonic index is easily explained. We know from previous investigations that in the same infection the leucocytes may in one case show a positive and in another a negative chemotaxis. The author himself formerly held the view that small amounts of dissolved bacterial bodies acted upon leucocytes in a positive chemotactic manner, but that larger amounts might act negatively chemotactic. This view had been accepted by him for the reason that his observations on guinea-pigs infected with typhoid or cholera seemed to permit of no other explanation of the phenomena. These views have been confirmed by Wright's valuable opsonin experiments, which have also shown that small amounts of the vaccine are generally able to cause a speedy and distinct rise in the opsonic index, but large amounts cause a considerable decrease. The same thing is observed in vaccination, for a vaccine is nothing else than bacterial bodies artificially killed. The great decrease of bacteriolysin, or opsonin content, after the introduction of bacteria, or vaccines, is analogous to the crisis-like decrease of the antitoxin content after an injection of toxin, the lowering being in no way related to the amount of toxin injected.

† The author has, for example, been able to show that in tuberculous foci entirely encapsulated by connective tissue the opsonic index was low, with a very decided cutaneous reaction, for the reason that the reabsorption of metabolic products of tubercle bacilli was absolutely impossible

means by which a too severe reabsorption of bacterial products may be prevented. In chronic infectious diseases, especially tuberculosis, the case is different. By prescribing rest in bed, lying, sitting, walking, and well-regulated work, the reabsorption of the metabolic products of the bacteria is controlled. For example, by prolonged rest in bed we may produce an encapsulation of tuberculous foci, which could not be accomplished were more active work permitted, with its consequent increased circulation. This method has long been employed by all intelligent physicians, but did not come into general use because insufficient reasons for the therapy could be given.

It is now possible to prove the value of such therapy by observing the opsonic index. The same observations show, furthermore, that there are cases in which the focus is so small or so encapsulated that recovery within cannot take place, because insufficient antigen (metabolic products of bacteria) finds its way into the circulation to stimulate the body to form reactive substances in amounts necessary to facilitate the healing of the process.

In such cases one may either facilitate the reabsorption of bacterial metabolic products by increased blood diffusion—this making the filter around the focus more permeable—or by the introduction of an antigen. The latter is designated vaccination therapy, the principles of which are discussed in a separate chapter.

Hæmolysins.

In the same manner in which bacteriolytic reactive substances (bacteriolytic immune bodies and amboceptors and bacteriolysins) are formed after the injection of bacteria, hæmolytic reactive substances (hæmolysins) are formed after the injection of blood-corpuscles of a different species of animal.

Hæmolysins have, in common with bacteriolysins, the inability to cause hæmolysis in themselves. They represent amboceptors in the sense of Ehrlich's theory, requiring an activation by a ferment (the so-called complement) in order to exert the action as indicated by their name.

Nature of Hæmolysis.—Hæmolysis is the separation of hæmoglobin from blood-corpuscles, the opaque blood solution thereby becoming transparent (lac colour). A hæmolysis of a blood solution may be produced at any time by bringing blood-corpuscles into an an isotonic solution—*e.g.*, by adding distilled water. By hæmolysis in the special sense, however, as the term is always used in immunity, we understand the conversion of the blood solution into a transparent colour in an isotonic medium by specific amboceptors.

Preparation of Hæmolytic Serum.—A hæmolytic serum is obtained from a rabbit by the injection (preferably intravenous) of 1, then $\frac{1}{2}$, then $\frac{1}{3}$ c.c., of washed blood-corpuscles —*e.g.*, those of a sheep. Three or four days after each injection blood is taken from the ear-vein, and the titer determined.

This is done as follows: The serum, obtained by centrifugalization or sedimentation, is inactivated for half an hour at 56° C. Various dilutions of this are made, as, for example, 1 : 100, 1 : 500, or 1 : 1,000. To 1 c.c. of a 5 per cent. emulsion of washed blood-corpuscles 1 c.c. of the serum dilution is added, and as complement (an activating ferment), 1 c.c. of a fresh guinea-pig serum, diluted ten times with physiologic salt solution. The whole is incubated at 37° C. for a half to one and a half hours.

Hæmolytic Experiment.—

Emulsion of blood-corpuscles
+ Hæmolytic immune serum
+ Complement
} constitute a hæmolytic system.

The term 'hæmolytic system' will be repeatedly used in connection with complement fixation, discussed later.

Controls necessary in the hæmolytic experiment are :—

1. A control to show that the emulsion of blood-corpuscles is not spontaneously hæmolyzed (by an isotonia or by an insufficiently fresh emulsion of blood-corpuscles).

2. A control to show that the hæmolytic immune serum does not hæmolyze without complement.

3. A control to show that, on the contrary, the complement is active in the presence of a hæmolytic immune serum, but does not act without the addition of such serum, perhaps because of the amboceptors spontaneously present in the serum of the guinea-pig. One c.c. of the emulsion of blood-corpuscles is, therefore, mixed with the complement, in which case no hæmolysis should occur.

Significance of Hæmolysins in Transfusion.—The direct practical therapeutic significance of hæmolysins is inconsiderable, and, on the whole, rather negative in sense. Experiments in hæmolysis show why the transfusion of sheep's blood is accompanied with such undesirable sequelæ that the practice of transfusion of all heterogeneous blood was long ago abandoned.

After transfusion of heterogeneous blood, fever, chills, and evidence of irritation of the kidney, with albuminuria and hæmoglobinuria, often arise. The body forms hæmolysins against the heterogeneous blood-corpuscles, which dissolve the latter, causing fever and frequently irritation of the kidneys, with hæmoglobinuria, due to the elimination of the dissolved blood-pigment. Aside from these phenomena, occurring as a result of the excretion of the heterogeneous albumens liberated by hæmolysis, the formation of hæmolysins by the injection of sheep's blood brings about the production of reactive substances, which are not without effect upon the body, as the formation of such reactive substances is usually followed by a decrease in weight.

For this reason we have discontinued the practice of transfusion in cases where a severe loss of blood necessitates a repletion of the circulation, and instead merely administer physiologic salt solution, in order that the heart may not be entirely drained, leaving it to the hæmatopoietic organs to reproduce the lost blood-corpuscles. Transfusions are indicated only in severe anæmia (pernicious anæmia, leucæmia, etc.), where the hæmatopoietic organs are either insufficient or, owing to the affection, are incapable of forming normal blood.

According to the above statements, only human blood should be used for transfusion in human beings, the methods being (1) direct transfusion from vessel to vessel, technically difficult, and not without danger, because of possible coagulation ; and (2) intravenous injection of blood previously defibrinated by shaking with glass beads.

The latter method seems wholly unobjectionable, yet it is applied far less frequently than conditions indicate.

Isolysins.—In transfusion of human blood isolysins must be taken into account. These are hæmolysins, produced in

many persons when blood of another individual, but of the same species, is injected. We have to deal here with a very extensive individual specificity, which may sometimes have to be considered, especially in repeated transfusions. In a single transfusion the isolysins need not be taken into account. Before repeating an injection the patient's serum must be tested for isolysins *in vitro*.

Application of Hæmolysins in the Treatment of Anæmia.—The author has for some time practised the therapeutic application of hæmolysins in obstinate simple anæmia. It is a well-known fact that such cases of anæmia may improve spontaneously after a loss of blood, owing to a reaction which takes place in the hæmatopoietic organs under the stimulus following the bleeding, and the composition of the blood may be improved at the same time.

A properly regulated stimulus may be exerted upon the hæmatopoietic organs by hæmolysins (produced by the injection of human blood into animals), and especially upon the bone-marrow. Compared with venesection, which has also been applied therapeutically in anæmia, this method has the advantage that the principal components of the red blood-corpuscles, split up by hæmolysis, remain in the body for the building up of new blood-cells. Experiments, however, have not yet led to a definite result.

The chief significance of hæmolysis is at present not to be found in their practical significance, but in the part they have played in ascertaining the second chief form of immunity—the laws of lysis.

CHAPTER VIII

Wassermann's method of complement fixation—Technique of the Wassermann reaction — Theory of the reaction of complement fixation in syphilis—Application of the complement fixation method for the diagnosis of other infectious diseases—Indirect sero-diagnosis by the injection of antigens—Summary of prognostic conclusions.

BORDET and Gengou believed that those reactive substances formed by the body as a result of infection might be demonstrated *in vitro* if the serum in question was added to the proper bacterial extract. Antigen and reactive products then come into contact, this being manifested by the fact that the combination acquires the property of attracting complement. Consequently, upon the subsequent addition of the hæmolytic system (red blood-corpuscles + hæmolytic immune serum), there being no free complement, hæmolysis does not take place. The absence of hæmolysis thus indicates that antigen and antibody (reactive substance) have combined.

The following outline illustrates fixation of the complement according to the theory of Bordet and Gengou :

1. Antigen (extract of typhoid bacilli).
2. Antibody (reaction products or substances formed in the serum of a typhoid patient by the action of typhoid bacilli).
3. Complement (fixed by union of 1 and 2).

Upon subsequent addition of hæmolytic system (sheep's blood-corpuscles + hæmolytic immune serum), no hæmolysis occurs because the complement has been utilized.

After it had been proved by numerous experiments that Bordet's idea was correct, the method was applied for the

determination of an unknown factor. The same technique was used with a known serum containing antibodies to prove the presence of antigens, and *vice versa*, for the determination of antibodies in an unknown serum by means of a known bacterial extract.*

Complement Fixation in Syphilis.—This method first became of clinical significance when Wassermann, proceeding from premises, applied the principle to a disease which, because of its frequency, is of the greatest importance. It had never before been thought possible to make a diagnosis in such a manner, because all attempts to cultivate the *Spirochæta pallida*, now generally regarded as the infectious agent of syphilis, had proved unsuccessful.

Instead of the bacterial extract, Wassermann used a watery extract of the liver of a congenitally syphilitic fœtus, which is usually so rich in spirochætæ that it may easily correspond to a bacterial extract.

By this method a degree of certainty was obtained in syphilis diagnosis such as only a short time before had been regarded as impossible.

The following principles have been evolved from innumerable experiments :

Diagnostic Significance of the Wassermann Reaction.—A positive outcome of the Wassermann reaction, when the Wassermann technique has been carefully followed, proves the presence of syphilis, since no other disease occurring in the temperate zones elicits a positive reaction.†

A negative reaction does not necessarily exclude the possibility of syphilis, since many cases, especially in the initial stage, react negatively, and a positive reaction may be made negative through mercurial treatment without the occurrence of relapses being prevented thereby. We are, there-

* In using this technique, it is necessary that the hæmolytic complement be such that it can be fixed to the bacterial antigen-antibody. This leads to the assumption that there is a uniform kind of complement.

† Such a reaction is observed in leprosy, in some cases of experimental trypanosomiasis, etc. The conditions in scarlatina are not as yet quite clear. Even taking into consideration the arguments of the strongest opponents of the specificity of Wassermann's reaction, we must acknowledge that the positive result in syphilis cannot be confused with the positive results in other infectious diseases.

fore, justified in assuming that the disease is present in these cases in spite of the negative reaction. In untreated syphilis the reaction, almost without exception, is positive during and after the secondary stage.

Prognostic Significance.—Our knowledge of the nature of the antibodies of syphilis is not sufficient to permit of definite conclusions. It may be possible, that, as in tuberculosis, variola, etc., the antibodies are present even after a complete clinical recovery; yet the majority of investigators hold that the prognosis is more favourable when the antibodies are no longer demonstrable in the serum.

Therapeutic Significance.—Wassermann has truly declared that in time practitioners will be obliged to depend on this reaction for their therapeutic conclusions. At present we only know that during treatment the antibodies may elude our attempted demonstrations—not, however, in all or even in the majority of cases—and that in patients thoroughly treated with mercury the number of cases in which antibodies are demonstrable is much less than in those not so treated. Furthermore, antibodies seem to be constantly present in paresis—so much so that the presence of this disease is supposed to be questionable when the test for antibodies is negative. Whether or not this would suffice to establish the axiom that a syphilitic must be treated until antibodies are no longer demonstrable need not here be decided. To be sure, the fact that that greatly to be dreaded sequela, paresis, occurs only in individuals who give a positive Wassermann reaction seems to support this view. However, there are many individuals in whom the reaction disappears only temporarily, if at all, after mercurial treatment, and proof is still lacking that paralysis develops only in cases insufficiently treated with this remedy. It may be that paretics are those in whom the reaction does not disappear or always recurs in spite of mercurial treatment. Attempts to destroy the antibodies in paretics by mercury are rarely satisfactory, and saturation of the patient because of a positive serum reaction may lead to most disagreeable results.

It is well known that many clinicians consider a positive

reaction an indication for inaugurating a mercurial treatment, while others take the opposite point of view. It is well that this is the case, for only empiricism can decide the question. The theory, however, is needed to point out to empiricism the points requiring special consideration. The following must be read with this in mind:

Should a Mercurial Treatment be applied with a Positive Wassermann Reaction?—A positive complement fixation is in itself no reason for beginning mercurial treatment, for the antibodies proved by the positive reaction (amboceptors) are to be regarded as a favourable rather than an unfavourable item. We know, however, that complement fixation is a comparatively coarse method, by which only the larger amounts of amboceptors can be demonstrated. It is not probable, therefore, that the Wassermann reaction would ever be negative in a syphilitic, even though small amounts of amboceptors (as in tuberculin) were demonstrable by it. We know, further, from experiences in vaccination therapy, particularly in tuberculosis, that large amounts of amboceptors are present in the serum only when metabolic products of the infectious agent are thrown continually in the serum from the focus.

For this reason, therefore, it is possible that a negative Wassermann reaction may be transformed into a positive one after some time has elapsed, as the negative reaction does not indicate the absence of antibodies, and even in the presence of small amounts of antibodies the reaction is negative. We may, therefore, conclude indirectly from a positive reaction that a relatively active syphilis is still present, maintaining a certain standard of the production of antibodies.

So much for the theory of the reaction. Whether or not it is advisable to treat with mercury a syphilitic with an active focus, as indicated by a positive Wassermann reaction, empiricism must and will show.

Technique of the Wassermann Reaction.

Technique.—The following constituents are needed for the reaction :

1. Antigen.
2. Patient's serum.
3. Fresh guinea-pig serum, as complement.
4. Hæmolytic immune serum.
5. An emulsion of blood-corpuscles (from a sheep).

Antigen.—A watery or an alcoholic extract of the liver of a syphilitic fœtus, a liver of a guinea-pig, or of a human heart, is used as antigen. To one part (by weight) of the finely divided organic substance nine parts of alcohol are added, and diluted fivefold with physiologic salt solution. Each antigen must be repeatedly tested for its activity. Such antigens are now purchasable in the market.

Patient's Serum.—The serum required (1·5 to 2 c.c.) is obtained from 8 or 10 c.c. of blood, easily removed from a vein. When the blood is clotted the serum separates, and is drawn off and heated for half an hour at 55° C. (inactivated).

Complement. — Blood is taken from the carotid artery of the guinea-pig and centrifugalized. One part of the separated serum is diluted with nine parts of physiologic salt solution ; 1 c.c. of this is used.

Hæmolytic Serum. — The hæmolytic serum is obtained by injecting a rabbit (preferably intravenously) repeatedly with ½ to 1 c.c. of washed sheep's blood-corpuscles. The hæmolytic titer of the serum is determined by the hæmolytic experiment (see p. 118), and repeatedly controlled. In this test twice the amount necessary to produce complete hæmolysis (as shown by the titer) is used, in order to make sure that the amount of hæmolytic immune bodies necessary for hæmolysis is present, so that the absence of hæmolysis may not be ascribed to a lack of these substances.

Emulsion of Blood-Corpuscles.—Fresh sheep's blood is shaken with glass beads for the purpose of defibrination. After centrifugalization the supernatant serum is removed with a pipette, and the remaining blood-corpuscles repeatedly washed with physiologic salt solution and centrifugalized. Last of all, 1 c.c. is emulsified in 19 c.c. of physiologic salt solution, and the 5 per cent. emulsion required for the test is thus obtained.

Controls.—A large number of controls is required for the experiment. The activity of the complement must be tested, as

must the titer of the hæmolytic immune serum, and, above all, it must be determined whether the patient's serum (0·5 to 0·8 c.c.) and the antigen (0·5 to 0·8 c.c.) do not in themselves cause an inhibition of hæmolysis. Finally, we must be sure that the antigen used in the test inhibits hæmolysis by fixation of complement with the serum of a syphilitic, but not with the serum of non-syphilitics.

After these controls, which are usually carried out together, the test itself is made.

Order of Testing in Wassermann's Reaction.—To 0·2 and 0·4 c.c. of antigen (1) are added 0·2 and 0·4 c.c. respectively of the patient's serum (2). To this 1 c.c. of the diluted complement (3) is added. The whole is put in the thermostat at 37° C. for twenty to thirty-five minutes, in order to enable the mixture of antigen and serum to fix the complement, which it does if the proper reactive substances are present in the serum.

The hæmolytic immune serum (4) and 1 c.c. of the blood emulsion (5) are now added, and, after shaking, the whole is put into the thermostat at 37° C. After a quarter, half, and one and a half hours, the reaction is noted.*

In the presence of substances reactive to the antigen (so-called antibodies) the complement is bound to the combination of antigen and antibody, inhibiting hæmolysis. The Wassermann reaction is then positive. Indirectly, we infer from fixation of the complement the presence of substances reactive to the antigen —*i.e.*, against the *Spirochæta pallida*—and from this we conclude that a syphilis infection has at one time taken place.

SCHEME ILLUSTRATING ORDER OF THE WASSERMANN REACTION—I.

Antigen.	Patient's Serum.	Complement.	Hæmolytic System.	
A.	S.	1 c.c. fresh guinea-pig serum in tenfold dilution	Twice the hæmolyzing dose of hæmolytic serum with the titer 2,000 to each 1 c.c. of the 1 : 1,000 dilution	5 per cent. emulsion of sheep's blood-corpuscle.
—	—	C.		
A.	S.	C.	Hæmolytic system	
A. +	S. +	C. + Hæmolytic system = (in absence of hæmolysis) a positive Wassermann reaction.		
A. +	S. +	C. + Hæmolytic system = (in presence of hæmolysis) a negative Wassermann reaction.		

* The tubes are not left for a longer time in the incubator, for the reason that the mixture of antigen, antibody, and complement is reversible—*i.e.*, can be split up—and a hæmolysis occurring at a later period would be of no value.

OUTLINE OF TEST OF SUSPECTED SERUM WITH CONTROLS.

Antigen.	Patient's Serum.	Complement 1 : 10 Dilution.	Hæmolytic System.		Illustrative Results.
			Hæmolytic Serum.	Sheep's Blood-Corpuscles.	
0·2 c.c.	0·2 c.c.	1 c.c.	1 c.c.	1 c.c.	+ + + = {Wassermann strongly positive} = strong inhibition of hæmolysis
0·2 c.c.	0·4 c.c.	1 c.c.	1 c.c.	1 c.c.	+ + + = ditto = ditto
0·4 c.c.	0·2 c.c.	1 c.c.	1 c.c.	1 c.c.	+ + + = ditto = ditto
0·4 c.c.	0·4 c.c.	1 c.c.	1 c.c.	1 c.c.	+ + + = ditto = ditto
—	0·5 c.c.	1 c.c.	1 c.c.	1 c.c.	− = {Wassermann negative} = hæmolysis
Hæmolytic System Control.					
—	—	1 c.c.	1 c.c.	1 c.c.	− = {Wassermann negative} = hæmolysis
Control of Activity of Antigen. (As controls, two positive and two negative sera.) Complement bound by specific serum. ,, not bound by normal serum.					
	Positive Specific Serum.				
0·2 c.c.	0·2 c.c.	1 c.c.	1 c.c.	1 c.c.	+ + + = {Wassermann strongly positive} = strong inhibition of hæmolysis
0·2 c.c.	0·2 c.c.	1 c.c.	1 c.c.	1 c.c.	+ + + = ditto = ditto
	Normal Serum.				
0·2 c.c.	0·2 c.c.	1 c.c.	1 c.c.	1 c.c.	− = {Wassermann negative} = hæmolysis
0·2 c.c.	0·2 c.c.	1 c.c.	1 c.c.	1 c.c.	− = ditto = ditto

If the reactive substances are absent, the complement is not fixed, hæmolysis occurs, and the Wassermann reaction is negative. (See also Appendix.)

Theory of Complement Fixation in Syphilis.

The theory of complement fixation assumed that the specific reactive substance in the serum of the syphilitic entered into combination with the syphilitic virus, thereby fixing the complement. This theory was prevalent as long as the liver extracts of a syphilitic fœtus were used as an antigen, but after it was shown that complement fixation occurred with other organ extracts, especially with heart extracts, the specificity of complement fixation was greatly doubted.

According to Much,* therefore, syphilis reaction by complement fixation is not a specific process. This author considers it a colloidal precipitation reaction between certain lecithin-like colloids of the extract and the globulins of the serum. The globulins in syphilitic serum and in the serum of certain other diseases are more unstable, and therefore cause a more intense precipitation.

Klausner's Test.—The result of Klausner's syphilis test is likewise associated with globulins—that is, with their increase. This test consists in covering the serum to be tested with distilled water. At the place of contact a turbidity, due to precipitating globulin, is noted.

It is one of the best-known facts of immunity that in certain infectious diseases the globulins are increased.

Specificity of Complement Fixation.—According to these statements, it is now a question whether complement fixation in syphilis may be regarded as a specific fixation. To be sure, Wassermann emphasizes the fact that liver extract of a syphilitic fœtus is still the best antigen, and that the only reason for using heart extracts is that there is not a sufficient quantity of the extract of fœtal syphilitic livers to satisfy the demand for antigen. Yet this does not alter the fact that in

* *Med. Klin.*, 1909.

from 95 to 99 per cent. of cases a heart extract (from a normal individual) gives similar results to those obtained with the extract of syphilitic fœtal liver.

The theory upon which this great discovery was based, and to which it is wholly due, is at present of uncertain value, and perhaps no longer tenable. In this respect the author has made the farthest advance, concluding from complement fixations with tuberculin as an antigen that complement fixation was a reaction of infection, and that its practical value was dependent upon the fact that syphilis cannot be easily confused with any other infectious disease.

Empiricism, an extensive clinical experience, has replaced the theory. The practical value of the Wassermann reaction has, nevertheless, remained the same, for it has been shown that the reaction is never positive in any other infectious disease if the prescribed technique is rigidly observed.

Thus the Wassermann reaction is a phenomenon the diagnostic application of which is now wellnigh indispensable.

Complement-Fixation Method in the Diagnosis of other Diseases.

It was quite natural that a method as successful as the Wassermann reaction for syphilis should be tried in other infectious diseases. However, no attempts in this regard have as yet been so satisfactory that any significance may be attached thereto.*

Diagnostic Significance in Tuberculosis.—Only in tuberculosis is the complement-fixation method worthy of special mention. A great many authors have busied themselves with investigations in this subject. Marmorek, who a short time ago startled, not only the medical world, but the laity as well with the announcement that he had found a new diagnostic for tuberculosis, did nothing more than apply the method of complement fixation. Wassermann thereupon explained that the reaction of complement fixation in tuberculosis was of no practical significance, since the biologic

* See also Appendix.

methods, which will be discussed later, fulfilled all that was required of them.

Nevertheless, the method of complement fixation is of scientific interest in tuberculosis researches, and its results afford us valuable hints in regard to the findings obtained by the biologic method of tuberculin diagnosis.

Wassermann and Bruck used tuberculin as an antigen, and assumed, in consequence, that substances entering into combination with tuberculin could be present only in persons who had been treated for a considerable period with tuberculin injections. His experiments confirmed this view. Wolff-Eisner was the first to assume that all derivatives of tuberculin were of the same nature and had a similar effect. Consequently, a great many investigators found tuberculin antibodies in the serum of tuberculous patients, who were, to be sure, under the influence of substances reabsorbed from their own tuberculous foci, but who had never been treated with tuberculin.

These statements differ somewhat in detail, probably owing to a difference in technique and the antigen applied. As a rule, the antibodies are found more frequently in advanced than in initial cases, although there have been instances in which it was shown beyond a doubt that positive fixations were present in healed or inactive tuberculosis.

Wassermann and Bruck's observations led them to the following conclusions:

1. Receptors for tuberculin are present in tuberculous foci. Tuberculin reactions, as fever and focal reactions, arise when tuberculin comes in contact with the receptors of the focus.

2. If such receptors are cast off and circulate freely in the blood, they unite with the tuberculin that has been injected. They act very much like antitoxins, and prevent tuberculin from reaching the focus; in other words, they form a blockade about the focus, and do not allow any tuberculin to approach. This form of immunity is present only when the casting off of such receptors is brought about by increasing doses of tuberculin.

Personal investigations of the author confirm the fact that the focal reaction arises if the tuberculin reaches the receptors

at the disease focus. They also indicate, however, that anti-tuberculin is an amboceptor, and for this reason the tuberculin reaction cannot fail, as Wassermann claims, if these amboceptors circulate in the blood. A tuberculin reaction—*i.e.*, fever and focal reaction—fails if the tuberculin injected is fixed to sessile receptors—in the connective tissue, for example.

Is the Injection of Antigens in Man and Animals—e.g., Tuberculin—to be regarded as a Sero-Diagnostic Method

In tuberculin diagnosis and in a number of other methods the serum of the patient is not examined, but toxins, as tuberculin, etc., are incorporated in the organism. This is none the less a sero-diagnostic method. This apparently contradicts the facts, but the reaction to the injected antigen affords information concerning the properties of the serum.

We are able to demonstrate the presence of bacteriolysins for certain bacteria (typhoid and cholera) by observing bacteriolysis directly (*cf.* Chapter VII. on Bacteriolysins). We know, also, that a direct observation of bacteriolysis of other bacteria—*e.g.*, streptococci—is difficult, and that in determining analogous reactive substances in the blood-serum (as bacteriolysins in typhoid fever), we are confined to indirect methods. *A comparison of bacteriolysis by means of these indirect methods shows that the same materials are demonstrable as are directly active in bacteriolysis.* Thus, comparative experiments with bacteria, in which the presence of bacteriolytic immune bodies may also be directly observed through bacteriolysis, result in the important conclusion that the antibodies demonstrated by the complement-fixation method, by the opsonin method, and by the biologic reaction, are all identical, and that these antibodies are lysins, or substances closely related to lysins.

One of the most fundamental facts of immunity was established with this discovery—viz., that the substances arising when bacteria or heterogeneous cells (even heterogeneous albumen in a broad sense) are taken into an organism are lysins.

The author has endeavoured for many years to prove the

existence of such a law, having learned by experiments* that a law of this kind is really the foundation of immunity —more important even than the fundamental law that the injection of any heterogeneous albumen leads to hypersensitiveness.

The various methods, however, are not of equal value for the titration of typical antibodies. The one with the fewest disadvantages, and the most sensitive for demonstrating bacteriolytic antibodies, is the Pfeiffer experiment; and this would show much smaller amounts of bacteriolysins if tenfold the fatal dose, as advocated by Pfeiffer, were not used for titration, but simply the quantity of bacteria necessary to produce death.

The opsonin test is a very delicate method of determining these substances. Nevertheless, the biologic proof of the presence of lytic substances in the blood-serum of the patient by the direct incorporation of the antigen—*e.g.*, of tuberculin—is without doubt greatly superior. Since the only object in introducing the antigen is to discover the presence or absence of such substances in the blood-serum of the patient, this method, though indirect, is nevertheless sero-diagnostic.

The Introduction of Antigens as a Biologic Sero-Diagnostic Method.—These truly biologic methods have advantages which justify the belief that a new era in diagnosis has begun, because sero-diagnosis is not a laboratory method, eliminating clinical work. On the contrary, the question whether or not certain substances are present in the serum is decided by the introduction of an antigen easily obtainable, the result being gained from the patient himself in a very short time. These methods offer no difficulties that would prevent the general practitioner from applying them. Only since their adoption have we been able to say that sero-diagnosis is in the hands of the practitioner. The practical results accomplished in only a few months' time promise great future development of the new régime.

Sero-Diagnosis in Vivo.—Pollen Test in Hay-Fever.—Although there is some discussion about the matter, it

* *Cf.* 'Heufieber' and 'Frühdiagnose und Tuberkuloseimmunität.'

appears unquestionable that the first sero-diagnosis *in vivo* was made in hay-fever—unconsciously, to be sure, the authors, from Blackley to Dunbar, believing that they were working with a simple toxin action. As a matter of fact, pollen albumen is a substance similar to tuberculin, to which certain persons react, these people being able to liberate from this substance, not toxic in itself, materials acting as poisons. These individuals possess what the author designates as lytic antibodies—*i.e.*, substances reactive to pollen products. The author regards the occurrence of a reaction to pollen albumen as a sign of the presence of lysins. We may mention incidentally in this connection that this method is of pre-eminent diagnostic and practical significance.*

Antibodies in Revaccination.—Pirquet afterwards tested the phenomena occurring in revaccination, concluding that the serum of vaccinated persons contained bodies altering the type of reaction after the first vaccination. From the altered reaction type a diagnostic conclusion may be drawn as to the character of the preceding vaccination (or variola affection). These observations, which are of little practical value, are mentioned only because Pirquet was induced thereby to virtually adopt the lytic theory, in opposition to his own previous theory of serum disease (see chapter on Hypersensitiveness), which theory was outlined in the discussion on hay-fever.

Tuberculin as Antigen in Tuberculosis, Cutaneous Reaction, etc.—The sero-diagnostic method with tuberculin as an antigen is of very great practical significance. It matters little in what way the antigen is incorporated, whether subcutaneously (Koch's method) or intracutaneously (Escherlich's method). The other methods of introducing tuberculi, especially Pirquet's cutaneous and Wolff-Eisner's conjunctival tests, offer nothing essentially new. The author drew the conclusion, from the course of the various tuberculin reactions, that the serum of those individuals

* Those interested in this subject are referred to the monograph 'Das Heufieber' (Lehmann, München, 1906).

showing a reaction contained bodies that liberated from the non-toxic tuberculin substances eliciting such reaction. This is the only explanation for a number of new and certain older experiments in tuberculosis immunity, found occasionally throughout literature.

This lytic theory is deduced from a study of actual conditions, and nowadays appears self-evident. By a brief mental survey of the numerous theories of tuberculin reaction, one may form an idea of the progress which has been made, since it can now be proved that the occurrence of this reaction is dependent upon the presence of lysins. Aside from this, it will easily be seen how important it was to confirm the theory that tuberculosis infection and tuberculosis immunity (a vast and, in spite of the researches of thousands, unexplored domain) are dependent upon the same all-controlling law—viz., that the introduction of heterogeneous albumen causes the formation of reactive substances (of a lytic nature)—a fundamental law of incalculable value to biology.

Every tuberculin reaction indicates, as we have seen, the presence of lysins in the serum of the individual. Nevertheless, the reactions vary in diagnostic significance. The difference is due to quantitative conditions—that is to say, the substances in the serum elicit a reaction now to smaller, and again to larger, amounts, depending upon the manner of introduction.

It is impossible to discuss here these processes, which are not yet thoroughly understood. Only the conclusions which seem from a practical standpoint the most valuable can be given.

Cutaneous and Subcutaneous Tuberculin Tests.—In subcutaneous (Koch), cutaneous (Pirquet), and intracutaneous introduction, not only those individuals with an active process react, but also individuals with an inactive and healed tuberculosis. In subcutaneous injections this is especially true of reactions to the repeated injection of tuberculin, formerly used unhesitatingly as a diagnostic test. On the other hand, a reaction after the first subcutaneous injection of tuberculin is indicative of an active tuberculosis.

Conjunctival Tuberculin Test. — A positive reaction following a conjunctival application of 1 to 3 per cent. old tuberculin solution indicates with as much, or even more, certainty the presence of an active tuberculosis. This method possesses the advantage over the subcutaneous method, that if all contra-indications are observed (avoiding reinstillations, especially instillations in diseased eyes that are, or have been, tubercular or otherwise diseased), there is no possibility of danger or injury.

The ability to react to tuberculin may disappear spontaneously in tuberculous individuals. This is a bad omen.*

Prognostic Significance of Results of the Tuberculin Test.—Even when a reaction to tuberculin is present, the reaction to the toxins liberated by the action of the lysins may vary. It follows in the main the laws governing the injection of heterogeneous albumen, and is thus represented by the phenomenon of hypersensitiveness. In the reaction of the body to derivatives of tubercle bacilli, either the inflammatory character (exudative form) or the formation of connective tissue (productive or infiltrative form) may predominate. As the encapsulation by connective tissue plays an important part in both complete and relative healing of tuberculous foci, it is evident that the productive form of inflammatory reaction may be regarded as the more favourable.

Owing to the great practical significance of a prognosis made by observing the mode of reaction to tubercle bacilli products, we may mention briefly the reports which, in the opinion of the author, are now substantiated. The great prognostic significance of this method is becoming more and more generally recognized. There was from the first no

* One might be inclined to attribute this absence of reaction to an absence of lysins. However, according to experiments of the author, and also according to experiments in complement fixation, lysins are present even in tuberculous cases showing a negative tuberculin reaction. The body has merely lost its ability to respond by reacting to the liberation of the toxin. The reaction designated by the author as ' rapid and weak ' is one stage of the body on its way to complete loss of reactive power. The loss of the power of reaction after tuberculin cures is a very different matter. The reader is here referred to Wolff-Eisner's article in ' Handbuch der Serotherapie ' (München, 1910).

question in the mind of the author as to the result of the controversy, since the tuberculin reaction itself shows the manner of reaction of the body to products of tubercle bacilli. By observing this we have a direct method of determining the manner of reaction at the disease focus.

Summary of the Prognostic Conclusions drawn from the Course of the Tuberculin Reaction.

A positive conjunctival reaction indicates the presence of an active tuberculosis. Although a favourable prognosis in an active tuberculosis is never justifiable, a strong conjunctival reaction is, other things being equal, more favourable than a weak reaction or none at all. This, of course, applies only when a tuberculosis is proved to be present, since otherwise a negative reaction indicates that there is no active process.

The same is true of the cutaneous normal reaction, by which is understood a local reaction following a vaccination with 25 per cent. tuberculin, and persisting for three or four days.

In cases of tuberculosis that have been confirmed beyond question clinically (and before a case is considered proved the presence of tubercle bacilli must, if possible, be shown), the lack of a conjunctival reaction must be regarded as an unfavourable prognostic sign. The same may be said of a negative cutaneous reaction and of that form running a rapid course which we have designated 'rapid reaction.' A failure of cutaneous reaction may be considered unfavourable even when the conjunctival test has been positive. This alone is ample proof that there are cases in which a positive conjunctival reaction does not justify a favourable prognosis.

A continuous form of cutaneous reaction occurs in clinically healthy persons, in those in whom a tuberculous process is healing, or in individuals in whom an active tuberculosis is present, but has continued for years.

The prognosis varies according to the clinical picture. However, it is favourable in any case of continuous reaction.

The indirect method of serum diagnosis from reactions

following the introduction of an antigen is naturally applicable only in case the serum of the healthy individual does not itself contain substances which would cause a reaction following such introduction. We have long known from the Pfeiffer experiment that bacteriolysins for typhoid and cholera bacilli are present in healthy persons. This is so invariably the case that we may consider immunity as only a quantitative increase of a normal condition. This explains how the introduction of typhoid or cholera antigen may cause a reaction even in healthy individuals. Such a reaction, however, does not justify the assumption that a change has occurred in the serum owing to a previous affection. It was because of this essential difficulty that Chantemesse's ophthalmo-diagnosis for typhoid fever, which was received with so much enthusiasm, proved a failure. The conditions in tuberculosis are usually favourable for the introduction of an antigen for diagnostic purposes. The tubercle bacillus and its derivatives resist lytic influences so strongly that a reaction between the lysins present under normal conditions and the tuberculin may be regarded as impossible. The many thousands of observations in the application of tuberculin show that in reality no reaction takes place in healthy subjects (man or animal), and that lytic substances, even if normally present, like those for typhoid, do not suffice to produce a reaction.

Practical Significance of Indirect Sero-Diagnostic Methods.—Therefore, even if the indirect sero-diagnostic method of antigen incorporation were limited to revaccination, hay-fever, and tuberculosis, the value of the method in practical medicine is still enormous, and is surpassed only by the Wassermann reaction in syphilis. Besides this, paths have been opened in the difficult subject of immunity. We may even hope that antigen inoculation has not yet reached the limit of its usefulness. It has already been proved that the method may be applied for diagnosis of bovine tuberculosis, and that the extremely difficult diagnosis of glanders (malleus) is also facilitated.

Possibility of Use of this Method in Syphilis Diagnosis.—It now seems probable that this method may in the future

be applied in preference to the Wassermann reaction. This would be advantageous for the reasons above described—primarily, because of its simplicity. This hope is based on the fact that a syphilitic responds to an inoculation with extract of syphilitic material—*i.e.*, an antigen, with a reaction differing so greatly from that shown by a non-syphilitic as to be unmistakable. In order that this method may be safely applied with a living subject, it is necessary that the antigen be so prepared that, without loss of activity, it will hold no danger for a non-syphilitic; in other words, to obtain a syphilitic extract which is killed, so that there is no danger of infection, and yet so carefully prepared that it loses nothing in efficacy.*

It was mentioned early in this chapter that the method of complement fixation, as well as the vital method of antigen incorporation, indicates as a rule the presence of lytic immune bodies. To be sure, it is possible to obtain results by the Wassermann method which are similar to those produced with the vital antigen reaction. Nevertheless, complement fixation will not supplant the latter method. Quite apart from the technical simplicity of the antigen reaction methods, they are exceedingly delicate, are capable of the finest modulations, and permit a much nicer discrimination in clinical diagnosis. It must be remembered that complement-fixation methods give positive results only when the reaction substances are present in the serum in comparatively large quantities. This explains why the reaction often fails in positive syphilitic cases, especially in initial cases where the reactive substances in the serum are not yet sufficiently abundant, or in old cases where they are inadequate. Only by assuming that this method fails in the demonstration of small amounts of reactive products, can we explain Wassermann's first contention that reactive bodies were present only in tuberculous patients who had been treated with tuberculin, a statement which seemed to contradict absolutely the unity of tubercle bacilli toxins now proved by Wolff-Eisner to exist. However, it soon

* Neisser (*Berlin. Med. Ges.*, 1908) claimed to have found such an extract, but has made no further report.

appeared that the complement-fixation method yielded results identical as a whole with vital antigen reactions, the only difference being that small amounts of reactive substances were not demonstrable by the former. Concurring investigations of various authors proved the fact, no longer disputed even by Wassermann, that positive complement fixation may be found, not only in individuals treated with tuberculin, but also in those in whom large amounts of tubercle bacilli products have been reabsorbed, and in whom a greater production of reactive bodies might be expected in evidence of a reaction of the organism to the stimulus.

It appears from the foregoing that the method of complement fixation and biologic antibody reactions—*i.e.*, reactions occurring after the introduction of antigens—indicate the same lytic reactive bodies; also, however, that the vital antigen reactions are far more delicate, so much so that they excel all chemical methods, permitting as they do the demonstration in the serum of reactive bodies in amounts which would elude chemical analysis. Nothing can better illustrate the value of this biochemical method than comparison with the more limited chemical analysis, highly developed as the latter is.

CHAPTER IX

Vaccine-therapy and effect of vaccination—Special application of vaccine-therapy.

VACCINATION was formerly often applied for immunizing purposes. The term, as everyone knows, is derived from cowpox vaccination, which, in spite of all our scientific progress, is still the most successful instance of protective vaccination that we have. Such vaccination was formerly known as active immunization.

It has long been noted that during an epidemic disease vaccinated persons show, under certain conditions, a tendency to the disease. This is what Wright calls the 'negative phase.' Because of this negative phase, passive immunization has often been combined with the active form, thus obtaining the advantages of both active and passive immunization, and avoiding the disadvantages of each. Such vaccinations have been successful in typhoid fever, cholera, and plague, but not overwhelmingly so, especially in diseases affecting man. The reason for this is not quite clear. For that matter, the etiology of typhoid itself is not yet well defined. It is probable that in those persons who are immunized against the disease the bacteria are still able to establish themselves and propagate in parts protected from the bacteriolytic power.

Indications for Vaccine-Therapy.—Prior to the researches made by Wright, vaccination was seldom used for therapeutic purposes; for a vaccine is a more or less modified poison, and it seemed inconsistent to inject additional poison into the body which was already struggling against

such substances. However, this is only partially true. *There are chronic infectious diseases in which healing fails to take place, for the reason that the body is not strong enough to react.* An instance of this is found in leprosy. There are other examples more familiar to the practitioner—*e.g.*, tuberculosis. In certain forms of tuberculosis, as in skin diseases acquired through contact infection (lupus, scrofuloderma, and the like), healing cannot occur for the reason just mentioned. It is the author's opinion that the other forms of tuberculosis also develop, for the reason that the bacteriolytic reaction of the body occurs too slowly, and therefore too late, to destroy the tubercle bacilli occupying the centre of the tubercle.

If we regard tuberculin, not as a toxin secreted by tubercle bacilli, but as a body substance of the bacilli acting as a toxin, we may say that vaccination-therapy has been applied for a long time—in fact, since Koch's discovery of tuberculin.*

Significance of the Negative Phase.—As has been mentioned, a negative phase following the inoculation of bacterial substances has long been a recognized fact. This is clinically manifest by an increased susceptibility to infection of the vaccinated person. This was a matter for serious consideration, in view of the rapid spread of vaccine-therapy.

The great service rendered by Wright and his school consisted in showing by means of the opsonic index a phase never before demonstrable. A negative phase occurs after practically every vaccination. The larger the dose, the more intense is this phase, and the longer its duration. It is a great mistake to undertake a revaccination during this phase, as the protective forces would thereby be lessened rather than increased (see p. 116, footnote). Hence vaccination for immunizing or therapeutic purposes should be attempted by no one who has not learned by opsonic experimentation to follow intelligently the effect of the inoculation of bacterial products; and he who does use these substances, which, independent of the amount of the dose, affect different

* Wright also speaks of tuberculin injections as 'vaccine-therapy.'

persons so differently, without familiarizing himself with the subject, commits a very grave error.

Without underestimating the disadvantages and mistakes of the opsonin technique, the author must admit that at the present time there is no other method enabling one to obtain an equally good result.

Vaccine-Therapy without Determination of the Opsonic Index.—Exception might be taken to our view on the ground that Wright himself no longer regards the opsonic index as indispensable in vaccine-therapy, and that we have stated earlier in this work that the opsonic index proved nothing (especially in tuberculosis) that might not be learned by carefully observing the clinical course, and determining the sensitiveness to tuberculin by means of the vital antigen reaction. However, the above statement applies only to tuberculosis, and even here we regard the determination of the opsonic index as a control of the clinical finding—troublesome, to be sure, but worth the pains, and an invaluable means of gaining an idea of the effects of the tuberculin treatment. Wright's repudiation of the opsonic index appears at first thought inexplicable, in view of the fact that this was the essential one of the new factors which he introduced into the old method. He it was who designated the injection of bacterial products for therapeutic purposes as vaccine-therapy. His stand is explained only when we consider the fact that because of its difficulty the determination of the opsonic index can never come into general use among practitioners, *and that his insistence upon it stood in the way of a general application of the vaccine-therapy*. This factor must have been a decisive one for Wright—that he should renounce the investigations of half a lifetime.*

It must be conceded—a fact which the author has contended from the first—that anyone experienced in opsonic work may practise vaccine-therapy even without knowing the course of the opsonic curve. However, we still adhere to

* The recommendation of vaccines prepared under the supervision of Wright himself contradicts his view that, if possible, every patient should be treated with vaccines prepared from the bacteria of his own foci (auto-vaccines).

our view that for those wishing to apply vaccines therapeutically the knowledge of the course of opsonic curves affords a groundwork upon which vaccine-therapy may later be built from a clinical standpoint. We are, therefore, unwilling to sound the retreat at the first signal given by Wright—the more so since this would be in direct opposition to that author's previous scientific work. That Wright does not regard his former investigations as obsolete is shown by the fact that he has recently published his works on this subject in both English and German.

If vaccine injection is not permissible during the negative phase of the opsonic index—and, as we have seen, this is one of the principles of vaccine-therapy—it follows that the application of vaccines is contra-indicated in all infectious diseases in which 'bacterial poisons,' so-called, appear spontaneously in the circulation. This precludes all possibility of applying vaccine-therapy in most acute infectious diseases.*

At present there are practically only two classes of cases in which vaccine-therapy is indicated: *acute* and *chronic infections*. Vaccination is applicable in acute infections only when they are so localized that it is safe to assume that healing will not take place, the substances entering the circulation from the local foci being insufficient to allow reaction products (bacteriolysins, in the opinion of the author) to reach the circulation in adequate amounts. Such a condition is possible in various diseases: in furunculosis, acne, and favus; in a number of cutaneous affections; in localized, particularly in relapsing, suppurations; perhaps also in gonorrhœa and erysipelas. The results obtained with vaccine-therapy in occasional cases of cholecystitis and colicystitis are explained in the same way. Wright assumes, further, that under certain conditions the reactive substances do not come in sufficient contact with the disease focus—*e.g.*, in cases of deficient circulation. Careful investigations, how-

* The only hope of a further modification of the vaccine-therapy in infectious diseases is contained in the recent findings of Wolff-Eisner—viz., that one kind of vaccination (intracutaneous injection) causes the production of fixed receptors, which are able to fix bacterial poisons and prevent their absorption by the central organs.

ever, have not been made in the last-named diseases, especially in regard to the behaviour of the opsonic index in the course of the disease.

Vaccine-Therapy in Acute Infectious Diseases.—In most of the acute infectious diseases not mentioned here we cannot expect especially good results from vaccine-therapy. In typhoid the character of the opsonic index would require a special study. In ulcus serpens, which is a local pneumococcic infection, we might perhaps expect results from vaccine-therapy, for the reasons above mentioned.

Vaccine-Therapy in Chronic Infectious Diseases.—The conditions in chronic infectious diseases are more favourable for vaccine-therapy. In leprosy, actinomycosis, glanders, and chronic sepsis, although a vast amount of work has been done, particularly in opsonic studies, nothing of value has been accomplished. Most investigators have occupied themselves with the study of conditions in tuberculosis, and vaccine-therapy finds here indeed a rich field. Cases of local tuberculosis, lupus, etc., can be treated in this way, and, moreover, an exacerbation of the infection may be prevented by keeping the opsonic index at a high standard through vaccine-therapy in cases showing a partial healing of the focus.

In the majority of cases of active tuberculosis, however, vaccine-therapy, according to Wright's principles, is not justifiable, because, as may be seen both by the clinical symptoms and the fluctuation of the opsonic index, toxins from the focus are already reaching the circulation in too large amounts and vaccination only increases this quantity.*

It is Rothschild's recent belief that many failures of the tuberculin-therapy are attributable to the fact that auto-tuberculin has not been utilized for therapeutic purposes. This view is similar to that of Wright, who advocates the use of auto-vaccines in other infectious diseases. Nevertheless, it is very unlikely that the results thus obtained would be much more remarkable—quite

* The employment of vaccine in the effort to produce fixed receptors is somewhat different. Although the technique in some respects is similar, the production of fixed receptors, as proposed by the author, is entirely different from Wright's theory which is that the object of the therapy is to increase the opsonic index.

apart from the identity of bacteriolysins, accepted by the author and supported by much evidence—for the reason that individuals who auto-tuberculinize themselves spontaneously run a course anything but favourable. If auto-tuberculin were the true remedy, there would be less to fear from tuberculosis, since the lesions would heal spontaneously after being activated in this manner.

The auto-tuberculin therapy has long been used by English authors in a much simpler manner than Rothschild's—viz., by prescribing rest and exercise under the control of the opsonic index, whereby the spontaneous production of tuberculin is very well regulated.

It might be argued that auto-tuberculinization is unsuccessful, because the spontaneous production of tuberculin takes place irregularly, both as to time and quantity, or because the negative phase is not observed, etc. But this would imply that vaccination-therapy would be without result in all advanced febrile cases, in which tuberculin is always spontaneously produced. On the other hand, one cannot but admit that, according to the clinical symptoms and the elevation of the opsonic index, a reabsorption of tuberculin from the focus occurs in many initial cases which yet do not show especially favourable chances of recovery.

Effect of Vaccination.

Small amounts of bacterial poisons act toward leucocytes in a positive, large amounts in a negative, chemotactic manner. Small amounts raise, large amounts lower the temperature. Small amounts increase the content of antitoxin or bacteriolysin; large amounts cause a corresponding decrease.

Similar conclusions are drawn from a study of the opsonic curves. They may be summarized as follows:

Small doses of bacterial substance cause a rise of the opsonic index. Large doses cause the index to fall.

As a rule, those doses which cause an increase of the opsonic index are preceded by a fall of the index—*i.e.*, the negative phase. In large doses this lowering is very

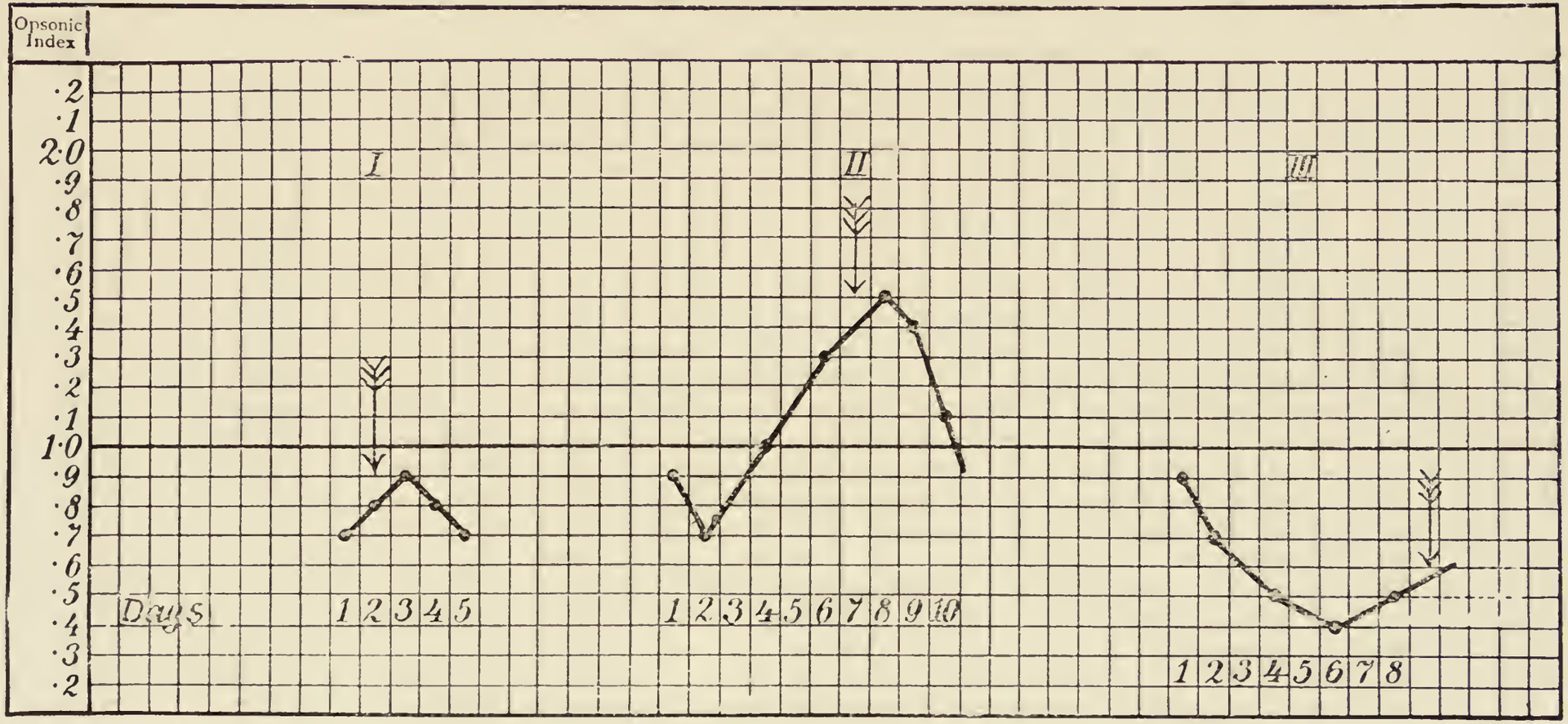
marked, and the injection may be followed by a continued fall.

These conditions are clearly illustrated in Matthews's three diagrams (*Lancet*, September 26, 1908). (*Cf.* curve on p. 147.)

The arrows show when a reinjection of vaccine is indicated for the purpose of producing a further increase of the opsonic index.

The behaviour of the opsonic curve is therefore partially dependent upon the amount of vaccine injected. However, it is not altogether dependent upon the dose, but rather upon the reaction of the organism to the vaccine. Notwithstanding the danger of being considered a neo-vitalist, the author counts it an honour to have pointed out this vital factor, particularly in the treatment of tuberculosis. Here again we have to deal with the much-abused term 'hypersensitiveness,' the general principles of which have been discovered, although its primary law is still unknown, because we do not know the substance eliciting the phenomenon. The expression 'anaphylactic body,' used by French authors, conveys no more meaning than the term 'lysin,' adopted by the author, since it constitutes only one of the factors participating in the origin of hypersensitiveness.

The reaction elicited by an injection of vaccine follows no hard-and-fast law, from which we infer that the amount of vaccine and its increase differ in each individual case. We find similar conditions in other fields, which are regarded as well understood. We know that after the incorporation of toxins, blood-corpuscles, or other substances eliciting lysin production, the antitoxins or hæmolysins may suffer a tremendous decrease—not at all in proportion to the amount of substance injected or the amount which the substance is able to fix. Similar processes take place when an enormous increase in antibodies (antitoxins or lysins) follows the incorporation of small amounts of antigen. Ehrlich's lateral-chain theory explains the latter, but the reason for the disappearance of the antibodies after the injection of the antigen is not yet known. However, the fact itself, now well established, quite without regard to the opsonic curve, shows from the standpoint of immunity the reason for



I. Course of opsonic curve after a small dose of vaccine.

II. Course of opsonic curve after a medium dose of vaccine.
Brief decrease, followed by a decided rise of the curve (therapeutic dose).

III. Course of the opsonic curve after a large dose of vaccine.
Lowering of the curve.
The arrows indicate the time most favourable for injecting a dose of vaccine.

proceeding with extreme caution in vaccinations. As a matter of fact, minimum doses of tuberculin were applied by numerous authors before the opsonic curve was known.

Special Application of Vaccine-Therapy.

Following these general remarks, we may give a brief survey of the general application of vaccine-therapy.

Preparation of Vaccines.—Vaccines are prepared as follows: By vaccine is understood a fine emulsion of a bacterial culture (killed by heating for an hour at 60° C.), and diluted with a physiologic salt solution to which $\frac{1}{4}$ per cent. of lysol has been added. Its sterility is proved by the culture method. The bacteria growing sparsely, as pneumococci, streptococci, and gonococci, the bouillon culture is applied instead of the emulsion. An emulsion is made uniform by shaking for from fifteen minutes to an hour in an apparatus devised for the purpose. It is well to enclose two or three sterile glass beads in the capsule, as this makes the emulsion more delicate and uniform, especially if the bacteria are difficult to separate, as are streptococci. The number of bacteria is previously ascertained, in order to establish the standard value, either by the plate method or by a method devised by Wright, which is as follows: The bacterial emulsion is mixed with equal parts of blood in an opsonin pipette, and the number of bacteria approximately determined, after counting out the blood-corpuscles and bacteria in the smear preparation. The formula is as follows:

$$\left.\begin{array}{l}\text{Number of bacteria}\\ \text{in 1 c.c. of the}\\ \text{emulsion}\end{array}\right\} = \frac{\text{Number of bacteria counted} \times \text{number of erythrocytes in 1 c.c. (5 billion)}}{\text{Number of erythrocytes counted.}}$$

Stock vaccines, to be had in the market, are to be distinguished from true vaccines, made according to the same technique from the patient's own foci. Theoretically, the latter are to be preferred in many cases, but for practical purposes, so far as use in small hospitals and in private

practice is concerned, commercial vaccines may be used almost exclusively.

The following are the most important vaccines at the present time:

1. **Typhoid Vaccine.**—Formerly used for prophylactic purposes only.* Two injections should be given at intervals of ten days.

The first injection is made with 1 c.c. of vaccine and 1 billion typhoid bacilli of a twenty-four-hour culture, killed by heating to 60° C. Two billions are used at the second injection.

The activity of the preparation is limited to six months. Preventive vaccination with this vaccine causes headache, prostration, etc.

2. **Coli Vaccine.**—Polyvalent coli vaccine, from coliperitonitis, colicystitis, colisepis and intestinal colostrum.† One c.c. contains 10,000,000 or 20,000,000 of twelve-hour culture of colibacilli. This vaccine is applied in colisepsis, cholecystitis, colipyelitis, colicystitis, and coliendometritis. The treatment is begun with 15,000,000 bacilli, and increased weekly until it reaches 30,000,000.

3. **Staphylococcus Vaccine.**—This consists of various species of staphylococci, twenty-four to forty-eight hours old. The commercial vaccine is prepared in three degrees of strength, 1 c.c. containing 100,000,000, 200,000,000, and 500,000,000 staphylococci respectively.‡

This vaccine is used in the treatment of furunculosis, acne, staphylococcic sycosis, and other local staphylococcus infections. In the annoying affection comedones, with superficial pustules without the formation of scars, good results are often reported, even after the first injection. In acne with deep, nodular scars, persisting for years, a long period of vaccine treatment is required, preferably with auto-vaccine. In furuncles the following is advised: In

* Parke, Davis and Co., London.

† Kaiser Friedrich Apotheke, Berlin

‡ Parke, Davis and Co.; Chemische Fabrik Güstrow—'Opsonogen'; Kaiser Friedrich Apotheke—polyvalent staphylococcus vaccine from cases of furunculosis, mastitis, osteomyelitis, and sepsis.

general furunculosis, ascending doses should be applied for twelve weeks. In isolated furuncles 1 c.c. of vaccine, containing 100,000,000 of staphylococci, is given at the first injection. In three or four days the dose is increased to 250,000,000 or 300,000,000. In a relapsing case the doses should be smaller, beginning with 50,000,000 staphylococci, and increasing the amount weekly, then at longer intervals, until the final dose of 500,000,000 is reached.

Good results have been reported from the vaccine-therapy in osteomyelitis and panaritium.

In chronic eczema (impetigo and sycosis) the vaccine-therapy should be long-continued, and perhaps used in connection with X-ray treatment and depilation.

The author uses still more caution in the treatment of localized suppurations (mastitis, osteomyelitis, etc.), beginning with 100,000 germs. Since the result is favourable, a rise of the opsonic index taking place and clinical symptoms appearing after the injections (slight headache, drowsiness, and indications of excitement), and since the final results are good, the method may be recommended.

4. **Streptococcus Vaccine.***—This is polyvalent streptococcus vaccine from cases of sepsis, panaritium, and peritonitis. It contains in 1 c.c. 5,000,000 and 10,000,000 streptococci two or three days old, originating from erysipelas streptococci. The stock vaccines require further dilution before using. The preparation is especially useful in localized streptococcus infections, as lymphangitis, erysipelas, and phlegmon, and more especially in mixed infections of streptococcus in tuberculosis.

According to the literature, vaccinations with pneumococci, *Bacillus Friedlander*, *Micrococcus catarrhalis*, and others, have been successful.

5. **Gonococcus Vaccine.**†—One c.c. of the vaccine contains 5,000,000 or 10,000,000 gonococci killed at 60° C. In gonococcal infections the commercial vaccine should almost always be used rather than auto-vaccine, which is often hard to segregate and is sometimes unobtainable. Generally

* Parke, Davis and Co., London; Kaiser Friedrich Apotheke, Berlin.

† *Ibid.*

speaking, vaccination has as yet been applied only in chronic gonorrhœal arthritis, in which gonococci can no longer be cultivated from the patient. According to the literature on the subject, the results in these cases vary somewhat. The treatment is begun with 2,500,000 bacilli, and increased to 5,000,000 and 10,000,000.

Allen reports good results in gonorrhœal conjunctivitis. His experiments were made first with commercial and later with auto-vaccines. Should his favourable results be confirmed, the vaccine-therapy might be tried in the treatment of gonorrhœa, since the conditions are the same in gonorrhœal conjunctivitis as in the acute form of the disease.

6. **Tuberculin Vaccine.**—The vaccine-therapy in tuberculosis is nothing more or less than a tuberculin treatment for which Koch's bacillus emulsion is used, this being of no essential importance. The principle of the method consists in using the minimum dose, which is not essentially increased during the treatment.

The author's experimental observations led to the application of the minimal dose, and he regards it a mistake to change to the maximum dose. Wright has adopted the same therapy by reason of his observations of the opsonic index.

Wright applies the therapy, particularly in chronic localized afebrile tuberculosis.

One c.c. of Wright's tuberculosis vaccine contains $\frac{1}{5000}$ to $\frac{1}{2000}$ milligramme of ground tubercle bacilli, while 1 c.c. of the commercial emulsion of the Höchster Farbwerke contains 5 milligrammes.*

To avoid errors, the quantities of the dilution of the bacilli emulsion (which contains 5 milligrammes of bacterial substance to each c.c.) are given below.

The corresponding dilution for each c.c. is :

1 : 200,000 = $\frac{1}{40000}$ milligramme.
1 : 100,000 = $\frac{1}{20000}$ milligramme.
1 : 20,000 = $\frac{1}{4000}$ milligramme.

* The vaccines, put up in separate doses, are for sale by Parke, Davis and Co., London, and Kaiser Friedrich Apotheke, Berlin. (New tuberculin emulsion, Wright Series.)

In applying this vaccine, the following principles may be derived from the investigations made by Wright's school:

1. Let us assume that we are dealing with a small, strictly localized focus, as, for example, an adenitis or a tuberculous arthritis. We may begin with $\frac{1}{20000}$ milligramme, repeating the injection at intervals of eight or ten days, and finally increase the dose to $\frac{1}{4000}$ milligramme monthly. In tuberculous arthritis we may also produce auto-inoculation by applying the congestion-bandage, by massage, etc.

One may also begin with only $\frac{1}{50000}$ milligramme in these cases. This is especially recommended in cases of lung involvement.

2. In dealing with non-localized tuberculosis, continuous doses of $\frac{1}{20000}$ milligramme are given without increasing the amount.

In tuberculous skin affections, such as lupus, scrofuloderma, or tuberculides, $\frac{1}{4000}$ to $\frac{1}{2000}$ milligramme is given.

In primary or secondary tuberculous cystitis, or in tuberculous affections of other parts of the uro-genital system, $\frac{1}{15000}$ to $\frac{1}{50000}$ milligramme is applied. Especial care should be taken to see whether a mixed infection with bacteria coli is present. If so, coli vaccine should be applied in addition. Good results are reported from this therapy, especially in tuberculosis of the bladder. This is manifest, even without observing the opsonic index, from the subsidence of tenesmus and of the pain, and from the diminution of pus.

In tuberculosis of the ears, nose, and throat, operative measures should be taken only when the opsonic index has been reduced to 1 by vaccine-therapy.

CHAPTER X

Active Immunization—Passive Vaccination—Chemo-therapy.

Active Immunization.

An animal may be actively immunized against poisons of a toxic character, as well as living bacteria. We shall first discuss very briefly immunization against toxins.

Results of Toxin Injection.—Three terminations are possible in the treatment of animals with toxins. They are as follows:

1. The animal dies.

2. The animal becomes insensitive to the action of the poison. This insensitiveness is acquired by formation of substances in the serum which fix the toxin. The mechanism of this process (discussed in detail in the chapter on the lateral-chain theory) enables us to understand the reason that in cases of active immunity to toxins hypersensitiveness is always transmissible with the serum of the immune animal to the sensitive animal to which it is transferred (passive immunization).

3. A frequent termination, but one of which little notice has as yet been taken, is that in which the animal does not die, but becomes cachectic, and forms no antibodies.

It sometimes happens, in actively immunizing an animal, that a hypersensitiveness develops instead of an insensitiveness, even when a very high degree of antitoxic immunity has been, and still is, present (Behring). In this case the serum is rich in antitoxins.

Hypersensitiveness was discussed so extensively earlier in

this work that here we will merely refer the reader to the chapter on that subject, and lay emphasis upon the fact that no satisfactory explanation has ever been given for this form of hypersensitiveness to toxins—*i.e.*, the form in which an animal contains the antitoxins in his blood in large amounts, and yet suddenly becomes so sensitive to the toxin that it dies from a dose which an untreated animal would easily bear.

Immunizing with Toxoids. — Active immunization of animals, especially for the purpose of preparing therapeutic sera, is often accomplished with toxoids instead of pure toxins — *i.e.*, attenuated toxins which, in spite of their diminished toxicity, still possess the same immunizing capacity. Loss of animals is thus avoided. Expressed in the terms of Ehrlich's lateral-chain theory, toxoids are toxins, the toxophore group of which has been destroyed, the haptophore group remaining intact. The occupation and casting off of cell-receptors is almost the same with toxoids as with toxins. Ehrlich's figure, however, does not hold good throughout for toxoids, since, according to Bruck and others, at least a slight toxic effect is required to stimulate the cells to cast off their receptors.

Simultaneous Method.—A still safer method of active immunization may be carried out in a manner similar to that with toxoids by injecting mixtures of toxins and antitoxic serum in such proportions that a very slight excess of toxin remains in the mixture. This is known as the simultaneous method. With this method the serum-toxin mixture may be applied in one or two injections, made either at the same or at different parts of the body.

Active Immunization against Toxins.

Throughout this work stress has frequently been laid upon the fact that bactericidal immunity should be differentiated from antitoxic immunity.

Immunization against bacteria may be brought about with living, unaltered germs, with living but attenuated germs, or with killed bacteria, according to the method just described —viz., a simultaneous injection of bacteria and immune serum.

Immunization with Living, Unaltered Germs.—This method is not often applied, since it involves all the dangers of spontaneous infection. Children are sometimes purposely exposed to measles in order that they may pass through the disease with other children, and certain stock-raisers allow their animals to be exposed to infections in order to be sure that they are proof against disease. Following the same lines of reasoning, before better methods were known, infections with living, unaltered germs were used to induce a mild form of disease which would afford a high degree of protection against a severe form. This was based on the knowledge gained by experience that an intentional infection often runs a milder course than one that is accidental and spontaneous. This custom was followed more especially when the epidemic was in a mild form, since it was observed that a mild infection afforded, as a rule, the same degree of protection as a severe one. The earliest form of smallpox vaccination (variolation, practised originally in India, and from there introduced into England) was nothing less than infection with living, *unaltered* infection-exciters, according to this principle.

An immunization may be safely accomplished with living, unchanged germs if the bacteria are injected at points which are not involved by the disease. For example, cholera in human beings is specifically an intestinal disease, and therefore, if cholera vibrios are injected subcutaneously or intravenously in man or animal, a fairly high degree of immunity may be obtained without danger.

Immunization with Attenuated Germs.

Methods of Attenuating Bacteria for Immunization.—Immunization with attenuated germs is practised much more frequently than with living, unaltered germs. The attenuation may be accomplished in different degrees and by various methods—by chemical means, by cultivating at temperatures above or below the optimal limits, or by animal transmission. Each of these methods is often applied for practical purposes.

The most successful method of immunization used to-day

—smallpox vaccination — is based on animal transmission, human virus being attenuated in this manner. On this method also depends bovo-vaccination, in which human tubercle bacilli are incorporated in cattle, and, in consequence of the attenuation thus produced, become harmless to the animal, at the same time affording it more or less protection, not only against the human tubercle bacilli, but toward bovine tubercle bacilli as well.

Pasteur's vaccines I., II., and III., applied for anthrax vaccination, depend upon the attenuation of anthrax bacteria at different temperatures.

Vaccination against rabies is dependent upon the drying of the infectious germ, and in this way diminishing the virulence. The drier the material, the less virulent it is.

As a rule, however, vaccination with living germs, even though attenuated, is attended with certain dangers that ought so far as possible to be avoided, both with human beings and with animals, where the same immunization can be obtained with dead bacteria. We have no right to expose a person to any danger whatsoever except in extreme cases, such as a bite of a hydrophobic dog, where the chances are that without protective vaccination the patient will die.

Active immunization, as practised upon human beings (soldiers encamped in infected districts and the like), is, as a rule, accomplished with killed bacilli only. A high degree of active immunity may be obtained with killed cholera, typhoid, plague, and dysentery bacilli.

In animals, where the question is not so much the avoidance of danger as the creation of a high degree of immunity, the simultaneous method is often practised to reduce the danger—*i.e.*, bactericidal serum is injected simultaneously with living or altered germs. The serum injection may be made previous to the bacterial.

Active Immunization in Man.

The field of active immunization is greatly limited in human beings. To be sure, it is possible to carry out such immunization if sufficient caution is observed; yet, once

attained, the disease is practically overcome, since immune substances are formed only when a certain stimulus is exerted upon the body. For this reason, Ehrlich's recent assertion that the formation of immune bodies might be ascribed to the ingestion of food is apparently not altogether consistent.

It is impossible to immunize human beings actively against all infectious diseases, since immune bodies are formed only when an irritation is exerted upon the cells.

Toxin Immunization. — Active immunization against toxins is of very little value, because injections of toxins are so injurious that experiments along that line have not yet been carried out, and all efforts have been confined to producing disease in animals, thence transmitting the immune bodies so obtained passively to man.

Polyvalence of Species : a Difficulty in Active Immunization.—Aside from the fact that it is impossible to immunize man against all forms of disease, active immunization in itself affords no certain protection.

There are many reasons for this: In the first place, there are a great many infectious polyvalent species—*i.e.*, immunization with one species of streptococci does not afford protection against another variety—and it is technically difficult, if not impossible, to carry out an active immunization against all forms of streptococci.

Moreover, it is possible for persons or animals to die, in spite of an intense active immunization, on account of a modified manner of incorporating the disease-producing material, even in relatively small amounts. Rabbits immune to tetanus are protected against subcutaneous infections, but die after intracerebral inoculation (Roux and Borrel). A guinea-pig which has been actively immunized against cholera is not protected against an infection through the intestinal canal.

In Pasteur's immunization against anthrax the animals are protected with a fair degree of certainty against a cutaneous infection, but not against infection through the intestines (produced by feeding substances containing anthrax spores).

Local Immunity.—Owing to these facts, many authors regard immunity accomplished by active immunization as local—*e.g.*, Dieudonné.* The author regards this view as

* 'Immunity, Vaccination, and Serum-Therapy,' 4th edition, 1905, p. 80.

erroneous and contrary to the true nature of immunity. Active immunization is without effect if the serum does not reach the site of bacterial proliferation (or of toxic action) in a sufficient quantity or with sufficient rapidity, or if the intermediate fixed or mobile receptors do not succeed in keeping the toxic action from the distinctly vital organs. Thus, animals possessing a natural immunity are protected by fixed receptors against subcutaneous and intravenous tetanus injections. However, they are unprotected if the poison is injected directly intracerebrally, and thereby enabled to pass directly to the sensitive cells—*i.e.*, cells in the vital organs containing receptors.

Character of Natural Immunity.—The author was able to prove that the *measurable quantity of fixed receptors runs parallel with the degree of natural immunity*, and that animals without fixed receptors for tetanus toxin are the only ones in which the fatal dose is the same in intracerebral as in subcutaneous injections.*

Snakes are likewise immune to their own poisons by reason of fixed receptors, and are therefore not injured by bites of other snakes; yet they are sensitive to intracerebral injection of the poison.

Ehrlich, unlike Gruber, contended that the formation of antibodies took place only in the sensitive organs. The author proved that, on the contrary, antibody formation is difficult only when receptors are present in highly sensitive organs alone, as in tetanus in guinea-pigs; and that antibodies are formed readily when receptors are demonstrable in less vital organs. The author also called attention to the importance of the fixing ability of receptors of connective tissue for typhoid toxin and tuberculin especially.

In his recent work, 'Beiträge zur experimentellen Pathologie und Chemotherapie' (Leipzig, 1909), Ehrlich unconditionally adopts this view-point. He specifies the four following possibilities:

1. Receptors may be entirely lacking. The animal is then naturally immune, but a formation of antibodies is impossible.

* *Centr. f. Bakt.*, 1908, vol. xlvii., Nos. 1 and 2.

2. Receptors are present, but only in organs upon which the poison does not act or in organs of less importance. This is also a natural immunity, and immunization is especially easy.

3. The receptors may be distributed over many parts, and in organs sensitive or non-sensitive to poison. A relative immunity now exists, and the termination of the infection is dependent upon the manner of application. The requirements for antibody formation are given in this case, but immunization is not always easy.

4. The site of receptors may be confined exclusively to the organs sensitive to toxin. The organism is then markedly sensitive. A formation of antibodies is theoretically possible, but is in reality difficult.

In one point, however, Ehrlich's first view seems preferable—*i.e.*, an organ or a tissue in which receptors are fixed is never entirely insensitive to toxin. How the toxic action becomes manifest depends upon the property of the tissue. A toxin acting fatally in the respiratory centre will not even cause a visible irritation in connective tissue. We must therefore distinguish carefully between local and general irritation.

These conditions may be illustrated as follows:

Inject 5 milligrammes of tuberculin, the result being high fever, exhaustion, etc. In this case the tuberculin has acted centrally and its effect has been general. In another case it remains fixed to the receptors of the connective tissue, producing, perhaps, an erysipeloid inflammation. Although the toxic effect is more severe than in the previous instance, it is nevertheless localized, and of much less significance to the individual than the central action.

In the question whether central or local effects are elicited by poisons (toxins or antitoxins), the distribution of the fixed and mobile receptors plays a large part. If, for example, an animal is immune against tetanus toxin, this high content of mobile receptors (called antitoxins), is of no use, provided the toxin is brought directly into the vicinity of the vital centres supplied with receptors. This mechanism explains why an animal actively immune to cholera may

succumb to a peritoneal infection, while another not previously treated may be protected against the same infectious dose by the serum of the first animal.

In the first instance the mobilizing of the antibodies does not follow quickly enough, and before the protective forces of the serum can be mobilized the cholera vibrios have increased so greatly that later, when they are destroyed by these forces, the fatal dose is liberated.

The same explanation holds true of the fact that it is possible to produce a cutaneous reaction in children passively immune to diphtheria by means of diphtheria toxin. The antitoxins cannot be transported rapidly enough in the poorly vascularized skin to neutralize the toxin molecule. As the toxins are locally preponderant, a local toxic action is found, *even though thousands of antitoxin units may be circulating in the blood-serum.*

Repudiation of the Theory of Local Immunity.—All these examples have been cited for the purpose of showing that Dieudonné's assumption of a local immunity was incorrect. As a matter of fact, immunization fails to take place *either because the bacteria are so protected from the action of the substances of the serum that their increase is greatly favoured and their subsequent dissolution liberates large quantities of poison,* or because *certain unusual conditions have permitted the toxin to come in direct contact with receptors of sensitive and vital organs,* the prevention of which is the task of sessile and mobile receptors.

Active Immunization in Healthy Individuals.—We now turn to the indications for active immunization in healthy human beings. The methods under discussion are applied, when not attended with danger, to produce an active immunization of long duration; for while such immunity affords no absolute protection, it can be created by no other means—certainly not by passive immunization.*

Active immunization is practised in human beings against smallpox, plague, typhoid, and cholera. It is true that because of the fact above mentioned—viz., that bacteria

* If we compare these methods with a generally known method, we must bear in mind that the question is not one of variolation, but of a process similar to vaccination.

may proliferate in places more or less protected from the bacteriolytic forces of the body—immunization against these diseases has no infallible protective power. Nevertheless, it can be proved that there are fewer cases of disease among vaccinated than non-vaccinated persons, and also that the disease runs a milder course in the former, probably because the bacteria coming in contact with the serum are killed more readily in such cases than in persons who have not submitted to previous treatment.

This is the present state of the question. The statistics would be of little interest to the practitioner. On the other hand, in the question of immunization against plague, typhoid and cholera, Hahn advances an argument worthy of consideration. In these diseases, precaution in avoiding infection plays no small part. So long as vaccination is entirely optional, and not compulsory, those persons who take the precaution to have themselves immunized are especially careful in any event, and not so liable to become infected; hence, too broad conclusions must not be drawn from the mere fact of the lessened number of infections in such cases.

Active Immunization in Disease.—Aside from the specific treatment of tuberculosis, active immunizations have only recently been employed for the treatment of disease. This form of active immunization is now known as vaccination, which subject was discussed in a previous chapter. For the sake of preserving the connection, let it merely be stated that active immunization is contra-indicated in all septic processes—*i.e.*, in cases in which the organism is overloaded with poison, and, therefore, does not produce antibacterial substances in adequate quantities. In such cases the toxin injected for the purpose of immunization is only added to the toxins already present, and the ill-effect is thus intensified. Vaccine-therapy in these cases would be justified only if an antibody formation did not accompany the disease, but took place following the administration of vaccines—a condition which Much believes possible, but which has never been proved and does not seem probable. The theories of Pfeiffer, Radziewski, and others, show that the fatal termina-

tion of an infection is never brought about by an absence of antibody formation against the infecting organism.

Active immunization for therapeutic purposes is indicated more especially in localized disease processes in which, because of their localization—often in poorly vascularized tissue, inhibiting the absorption of toxins formed at the disease focus—the formation of antibodies is inadequate, since the stimulus necessary for such formation—the so-called *ictus immunisatorius*—is wanting. The following conditions are included in this class: furuncle, acne, dermatomycosis, cystitis, and localized suppurations of various kinds produced by many kinds of bacteria. The bacteria which are etiologically accountable for the disease must, of course, be used in the treatment.

Passive Vaccination.

After this survey of significance, aims, and limits of the vaccine-therapy, we shall discuss passive immunization first of all for protection against toxins, which is the very essence of serum-therapy.

According to Ehrlich, the antitoxic sero-therapy is the ideal form, because it is etiologic in the strictest sense of the word, and because the protective substances of the serum are perfectly harmless, and exert their action only against the invading organisms, without altering other tissues or organs, as do even the best pharmaceutical preparations. Nevertheless, this commendation of serum antibodies, though justified in itself, cannot receive our full endorsement. In the first place, it is impossible to eject the harmless immune bodies of the serum alone. They must necessarily be used in combination with a foreign vehicle—heterogeneous albumen—which, as we have seen, is not only unsatisfactory as a vehicle, but may even lead to serious sequelæ. Moreover, the protection thus afforded is of short duration.* The protective substances obtained from another animal are eliminated in a comparatively short time. The question

* Römer's recent statement that the antibodies injected may sometimes remain active for a long time does not contradict this.

now arises whether the injection of antitoxins would not, according to Ehrlich's lateral-chain theory, cause the formation of substances which would neutralize the antitoxins subsequently injected. This question has hardly been ventured heretofore, and lacks a satisfactory experimental solution, although the answer must constitute the fundamental principle for the prophylactic application of sera.

The antitoxic serum-therapy is applied in diphtheria and tetanus, and, to a certain extent, in ricin and abrin poisoning.

In diphtheria and tetanus the bacteria are in nowise altered by the antitoxin, but the poison secreted by the bacilli is fixed and neutralized by the mobile receptors, and is thus prevented from uniting with receptors in vital organs. This, however, does not appear to lessen the value of antitoxin-therapy, since the bacteria, after being deprived of their toxic effect, are apparently unable to injure the body.

A serum-therapy in which antitoxins are injected must be effective if the quantity of antitoxins is large enough. Unfortunately, in many cases—in tetanus, for example—the therapy is often unsuccessful. This is due to the fact that the antitoxin is able to fix free toxin, but is unable to separate the toxins already fixed to the cell receptors, and therefore, already united to, and acting upon, the cells.

The antitoxin-therapy should therefore be applied as early in the disease as possible. Upon this principle are based the new methods of serum-therapy, in which subcutaneous injections have been replaced by intravenous or intramuscular inoculations (Morgenroth), in order to accelerate the reabsorption of the antitoxins.

It is theoretically possible to set free by sheer massive action, as it were, at least a part of the toxins fixed to the cells by the use of antitoxins in enormous quantities. This is the basis for the most recent clinical methods for bringing about a recovery in severe and apparently doubtful cases of toxæmia by the application of huge doses of from 30,000 to 60,000 units.

In cases of failing cardiac action the simultaneous intravenous injection of adrenalin acts as a powerful stimulant, sustaining life until the injected serum has had time enough to fix the toxin.

Chemo-Therapy.

To Ehrlich is due the credit for having created chemo-therapy. To be sure, specific remedies were already known, such as the mercurial treatment of syphilis and quinine-therapy in malaria. These remedies are characterized by the fact that they cause little change in the body-cells, and yet greatly alter the infection exciters. On the other hand, it was proved by Koch's researches that corrosive sublimate cannot be used as a bactericide, since it acts on the body-cells more readily and more seriously than it does on the organism producing the disease; but, in spite of this, a concise knowledge of chemo-therapy is lacking.

The essentials of chemo-therapy consist in the selection of substances which, while acting effectively on the parasites, inflict the minimal injury upon the host thereof.

Ehrlich's procedure was consistent and perfectly logical. In his hæmatological studies he first determined the affinity of various parts of the cell for certain stains. He formulated the principles of specific staining, and from the properties and the chemical structure of the staining material he developed his lateral-chain theory, described elsewhere. In his studies in oxygen requirement of the organism he determined that reduction ability was a fundamental property of living protoplasm. This discovery later gave him unlooked-for information regarding the action of chemicals in their relation to processes taking place within the animal body. Ehrlich's discoveries in chemo-therapy are the acme of all his work, embracing as they do the efforts of a lifetime, and having, moreover, a practical value—that of freeing mankind from severe diseases, especially those which hinder colonization and the spread of culture.

The task of chemo-therapy consists also in selecting from an infinite number of substances those that will affect the course of an infection. The experiments made thus far have dealt chiefly with trypanosomatous and protozoan infections. From the compounds already at hand or synthetically prepared the best must be chosen and tested on many different kinds of animals. Especial care must be taken that

the difference is as great as possible between the therapeutic dose for destroying the infectious agent and the fatal dose.

Such therapeutic experimentation requires a tremendous financial support. The Speyer fund in Germany provides most generously for this.

Side by side with the old axiom, 'Corpora non agunt, nisi soluta,' to which not enough heed is paid in the study of immunity, Ehrlich places another—'Corpora non agunt, nisi fixata.'

Ehrlich also simplified a number of difficult problems by this simple principle: Substances that were fixed by parasites he called 'parasitotrophic'; substances fixed by the receptors of the organs he called 'organotrophic.' *

Our first duty, then, is to find drugs which are strongly 'parasitotrophic' and as little 'organotrophic' as possible. As corrosive sublimate and most disinfectants are decidedly 'organotrophic,' it is self-evident that they cannot be applied in infectious diseases *in vivo*.

Ehrlich regards sero-therapy—*i.e.*, treatment with antitoxins and other antibodies—as ideal, since these bodies are not in the least 'organotrophic,' but purely 'parasitotrophic.' They attack exclusively those substances for the destruction of which the organism has produced them. Such substances he calls 'monotrophic.'

Ehrlich is quite right in considering these antibodies ideal remedies, because they do not act injuriously upon the organism, as do most drugs. But two things must be kept in mind: first, that the antibodies cannot be injected alone, but only in media of heterogeneous serum, which in itself elicits serum disease; and second, that only antitoxins neutralize the toxins, whereas the much commoner lysins, in combination with complement, act as cytotoxins, and by lysis liberate the toxic content of the bacteria and cells, even albuminous substances (albumino-lysins), eliciting, in proportion to the quantity of bacteria present, the phenomena discussed in the preceding chapter.

* This, it would seem, does not take into account a fact confirmed by the author's personal experiments—viz., that besides the formation of receptors, the organs are able to bind toxins physically (by absorption) (*Centr. für Bakt.*, 1908, vol. xlvii., No. 42).

The number of substances with which we have to deal in chemo-therapy is as yet very small. There is only a limited number of active chemical groups—viz. :

1. *Group of Arsenicals.*—These are, in chronological order : arsenic acid, atoxyl ; the new substitution products—phenyl-arsenic acid, arsacetin, arsenophenylglycin ; and also antimonials—*e.g.*, tartar emetic.*

2. Certain azocolours : trypan red (Ehrlich and Shiga), trypan blue, and trypan violet (Mesnil).

3. Certain basic triphenylmethan colours : parafuchsin, methylviolet, pyronin, etc.

As regards the chemo-therapeutic action of these preparations, the following possibilities exist :

1. The substance may act *in vitro* and *in vivo*. From this we conclude that the parasite has receptors fitting the groups of atoms.

2. This substance may act in the animal body, but not *in vitro*. There are two explanations for this : in atoxyl, for example, even very high concentrations do not act bactericidally upon the parasites *in vitro*, while, according to Koch, trypanosomata are killed *in vivo* in a very short time. Ehrlich proved that *in vivo* atoxyl is reduced to para-aminophenyl arsenic oxide, a compound which kills trypanosomata *in vitro* also, even in the highest dilutions. The pentavalent arsenic rest of the atoxyl is transformed into the unsaturated trivalent arsenic rest, which possesses a greater avidity for receptors of the parasites.

Atoxyl (metarsenic acid anilid) is, as Ehrlich has pointed out, better adapted for destroying trypanosomata than arsenic. It remained for experimental therapy to find whether still more favourable results could be obtained by substituted atoxyl preparations. The substitutions were successful only after assuming the structure of atoxyl to be, not, as

NH-arsenic acid rest,

as formerly supposed, but

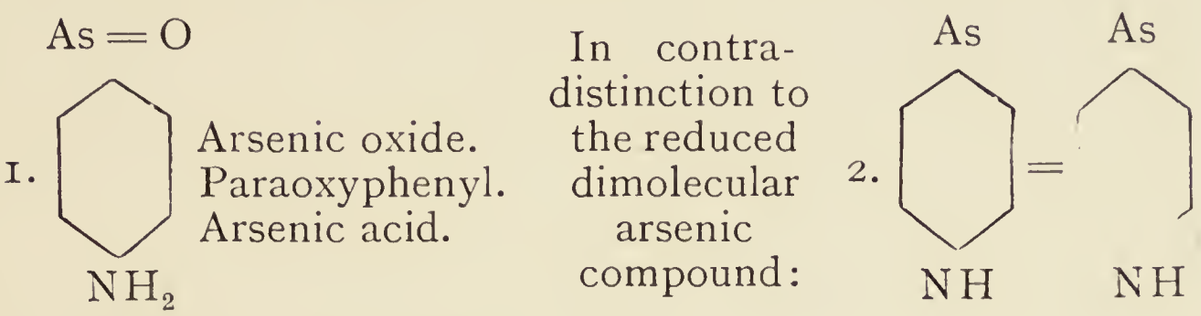
NH_2

Arsenic acid rest

* To these must now be added '606,' which is discussed in Chapter XI., p. 170.

—*i.e.*, as an arsenyl acid (similar to sulphanilic acid), which is far more adaptable to chemical substitutions. By substituting acetic acid in the molecule (acetylization), preparations were obtained which, like the usual substitution preparations of this character, showed the same bactericidal action with a twenty- to sixty-fold milder organotropism.

The action of the reduced arsenic oxide compound—



—can be seen from the following:

The former is trypanicidal in a dilution of 1 : 20 (paraoxyphenyl arsenic acid). In dilutions of 1 : 50 it is without effect, while the reduced molecular arsenic compound affects trypanosomata as follows:

In a dilution of	1 : 100,000	kills	immediately.
,, ,,	1 : 500,000	,,	in three minutes.
,, ,,	1 : 10,000,000	,,	in eighteen minutes.

The other possible explanation is: The therapeutic agent, such as pyronin, trypan red, or arsenophenylglycin, does not kill the parasites, but combines with certain parts of the protoplasm which possess the function of propagation. *In vitro*, therefore, the substance is without effect, but *in vivo* the prevention of propagation means death to the trypanosoma.

In many cases the chemical kills only a part of the parasites, whose disintegrated bodies elicit the formation of antibodies, bringing about the destruction of the remaining parasites. In this way, too, the action *in vivo* may be stronger than *in vitro*. For cases in which no chemical preparation capable of certain destruction of all the parasites is to be had, Ehrlich recommends that treatment be withheld until after the disease has reached a more advanced stage, in order that a sufficiently large amount of the parasites will

have been killed to furnish an adequate stimulus for antibody formation.

Immune Species of Parasites.

The action of drugs upon trypanosomata becomes less intense after a time, and the species becomes resistant—*i.e.*, immune to the action of the drug. This action cannot be attributed to cast-off receptors of the trypanosomata—trypanosomatous antitoxins, as it were—because the capacity of resistance to chemicals is inherited, and may be found unaltered after many generations. The reason for this resistance must, therefore, be sought in the trypanosomata themselves.

Ehrlich's explanation is that the species in question have cast off their receptors fitting this particular chemical, or else that the receptors have experienced at least a diminution of their avidity. The receptors become shorter in various stages.

The immunity of trypanosomata, their so-called drug-resistance, is a great impediment to therapy. However, we try to bring the arsenic into the vicinity of the parasite by means of other receptors of the trypanosomata, perhaps by an acetico-receptor—*i.e.*, a receptor adapted to the fixation of acetic acid. This is accomplished by fixing the acetic acid group in arsenophenylglycin.

Application of Chemo-Therapy in Man.

Since by painstaking animal experiments therapeutic agents destructive to parasites have been found, it is possible that the same substances may also be applied with human beings. In man, however, the substances must be used with much caution, for unforeseen complications sometimes arise, as, for example, in the eye after the application of arsenic preparations. The conjunctival and cutaneous reactions are frequently used, in accordance with Ehrlich's suggestion, before applying the drug, in order to find whether a hypersensitiveness be present.

If substances, active against parasites, have been found, the chances are that these substances will be active against still

other parasites. We apparently have to deal with group receptors common to various parasites, just as in immunity we have a group reaction.

Present Status of Chemo-Therapy in Practical Medicine.

Chemo-therapy has long been practised, as witnessed by the quinine treatment of malaria and the use of mercury in syphilis. More recently this field has been broadened by the addition of atoxyl treatment of sleeping-sickness and syphilis, built up on the theory of Ehrlich and his school.

Ehrlich's work has in the meantime been productive of a number of practical results, justifying the hope that progress along this line will continue.

It is true that arsacetin has not fulfilled our hope of ridding the body of the spirochæte of syphilis. However, it has proved effective in a number of more tractable diseases. According to recent reports, it seems to be superior to the arsenic preparations in the treatment of relapsing fever. Nägeli of Zürich has been able to induce recovery from pseudoleucæmia by internal administration of 0·05 c.c. Trypan red and trypan blue, which are supposed to have first been used in trypanosomiasis, showed, according to Mantteuffel and Uhlenhuth, a distinct influence upon many varieties of spirilla, and Nuttall recently succeeded by means of this substance in overcoming piroplasmosis, a widespread disease epidemically very prevalent among dogs, sheep, and cattle.

Alt has recently applied arsenophenylglycin in paresis. As we know, according to Wassermann and many others, the Wassermann reaction is constant here—*i.e.*, it is positive in 100 per cent. of cases. Alt has succeeded in causing the continual disappearance of this reaction in many cases by treatment with arsenophenylglycin.

CHAPTER XI

Ehrlich-hata '606' (Salvarsan) and its application in human pathology.

Salvarsan.

SALVARSAN, the extremely valuable preparation discovered by Ehrlich from innumerable animal experiments made in his chemo-therapeutic researches, was for some time regarded as the highest achievement ever reached in chemo-therapy; and even now, when the first stir of excitement has subsided, the discovery is still regarded as marking a climax in scientific research and an important step toward the high level which we may yet hope to attain. The discovery was made possible by the chemical formula recognized by Ehrlich in atoxyl. Chemically, Salvarsan ('606') is dioxy-diamido-arseno-benzol.

In Salvarsan, Ehrlich believed that he had proved the theory of therapia sterilizans magna, so far as syphilis was concerned; in other words, that he had succeeded in killing all the parasites in the body by a single dose of a chemical possessing only a slight affinity for the cells of the animal body, but a strong affinity for the micro-organisms.

Already Salvarsan has a history in which one may trace the course common to all great discoveries—first the stage of unquestioning excitement, then the reaction to the opposite extreme of unquestioning opposition. As is usually the case, the discoverer, Ehrlich, was as innocent in the one extreme as the other. As regards the underestimation of Ehrlich's work at present, while it is evident that not all the budding hopes first started by the discovery will ever blossom, it is nevertheless true, in spite of the variety of opinions on the subject, that the work is, and will remain, a model of scientific procedure for all time; that Ehrlich's chemo-therapeutic

studies have opened up a new pathway in medicine; and that this discovery will henceforth stand as a monument, marking a great advance in medical research.

The application of Salvarsan was the goal striven for by Ehrlich's therapia sterilizans magna. The spirochætæ, now generally regarded as the cause of syphilis, were to be destroyed at a single blow. This accounts for the first phase of the use of the preparation—*i.e.*, the incorporation of the maximum *dosis tolerati*. Since relapses occurred in spite of these large doses, the treatment was given in several fractional doses, and finally Salvarsan was combined with mercury.

Even though this combination may be commendable, it is inclined to complicate still more the difficult question of the activity of Salvarsan therapy and its most effective form. It can now be stated positively that the sterilization of the body from spirochætæ is not successful, since relapses do occur; nevertheless, it is a fact that its use is followed by a remarkable effect on all the manifestations of luetic processes, even in ulcerous, tertiary, or congenital forms. It seems certain that under the action of Salvarsan a large part of the spirochætæ are killed, and also that after its use the Wassermann reaction is often negative. The latter is apparently due to the mechanism of anti-anaphylaxis, the amboceptors—*i.e.*, the carriers of the positive Wassermann reaction—being fixed through the liberation of the spirochæte bodies, and thereby disappearing.

The Practical Application of Salvarsan.

Summing up the experiments that have thus far been made with Salvarsan, we find that it has been applied in subcutaneous, intramuscular, and intravenous doses of from 0·3 to 0·7 gramme. After subcutaneous and intramuscular injections, necroses seemingly occur in all cases. This was first ascribed to the alkaline reaction of the remedy, but as they occur in neutral and acid-reacting solutions of the drug also, they are clearly dependent upon the fact that a Salvarsan focus is created at the site of the injection, from which focus arsenical products are split off, exerting a toxic action

upon the surrounding musculature. It may be mentioned in this connection that these necroses, wherein the tendency to heal is very slight, are often apparent externally only months after the injection. After subcutaneous and intramuscular injections, arsenic is demonstrable in the urine for approximately twelve days, in necrotic places for months.

Subcutaneous and intramuscular injections are given in alkaline, acid, or neutral solutions, which are active, and, as already mentioned, lead to local necroses, apparently as an expression of the local toxic character of the preparation. Ehrlich warns us against acid solutions because of their extreme toxicity and the fact that they are difficult to absorb. They may therefore be used only in the form introduced by Kroxmayer—of oily suspensions rubbed up with paraffin and oil, the action of which takes place only when the acid action is counteracted by the alkalinity of the blood. All the Salvarsan solutions used for the injection must be entirely fresh, because destruction takes place very rapidly through oxidative processes.

In intravenous infusions the preparation is absorbed very rapidly, and after three or four days arsenic is no longer demonstrable in the urine. Because of the absence of complications and the controllable absorption, intravenous injections are now preferred by the majority of authors, and are, after a little practice, easily administered. In cerebral affections caution must be observed in the application of Salvarsan, particularly in intravenous injections; and while only 0·3 to 0·4 gramme are used for the usual intravenous injection, only 0·15 gramme should be used here. The fact that caution is necessary in these affections is explained by the mode of action of Salvarsan, as evidenced by the findings mentioned above.

Mode of Action of Salvarsan.

The action of Salvarsan apparently consists in killing the spirochætæ—or, at least, a large part of them. The amazingly rapid subsidence of syphilitic symptoms, which gave rise to the first burst of enthusiasm over the drug, attests this view, as do other clinical phenomena observed in

its use. After the quickly-acting intravenous injections particularly a brief period of fever, chills, headache, and nausea is often observed. Ehrmann (*Wien. Klin. Woch.*, 1911) calls attention to the fact that a strong Herxheimer reaction often follows the use of Salvarsan, which is explained in the light of the observations made above. Treupel has also found this general reaction to be the rule after intravenous injections. In reinjection the reaction is weaker in the majority of cases, apparently because at the time of the reinjection the number of spirochætæ present is small, and hence the spirochæte toxins liberated are in such small amounts that the hypersensitiveness to them is not displayed. Only in exceptional cases does the reaction after reinjection attain the strength of that following the first injection. For the rest, we may assume with Ehrlich that an *ictus immunisatorius* is exercised on the organism by the liberation of the spirochæte toxins, so that theoretically the application of Salvarsan is especially indicated in cases in which syphilis, running a mild course, does not of itself afford the body the required stimulus to immunization. The fact of the Wassermann reaction becoming negative is often produced as a favourable argument for the action of Salvarsan, yet this indication appears to be a doubtful one, since, as has been said, it is possibly due to the mechanism of anti-anaphylaxis—*i.e.*, to the fixation of immune amboceptors.

The clinical action of Salvarsan consists in that in manifest syphilis primary as well as secondary and tertiary manifestations disappear. Whether in the application of the drug for the treatment of primary affections further symptoms remain absent has not been satisfactorily determined. It is well known that relapses can and do occur after the use of the preparation, but as to the relation of these relapses to Salvarsan therapy, and especially their relation to mercury, experiments have not yet been numerous enough to justify an opinion.

Indications for the Use of Salvarsan.

Salvarsan may be regarded as indicated in malignant syphilis and in cases refractory to mercury. Naturally, each physician is justified in using the preparation according to

his judgment in other syphilitic manifestations. Other important indications to which further research must be directed are syphilitic sequelæ—paralysis, tabes, etc.—since experience has shown that these cases are not improved by mercury, but often aggravated. As contra-indications for the use of Salvarsan may be regarded aortic aneurisms, severe arterioscleroses, and cachexia dependent upon syphilis, since in these cases the ability of the body to react is wanting.

APPENDIX

Supplement to Hypersensitiveness (p. 37).

Friedberger and Hartoch* have worked out a theory that hypersensitiveness is only an albumen-antialbumen reaction, and have proved that during anaphylaxis a second injection is followed by a decided diminution of the complement, which in a passively hypersensitive animal ends in complete disappearance.

These interesting experiments again confirm the author's theory, advanced in 1904, that the phenomena occurring after the reinjection of albumen are attributable to lytic processes similar to hæmolysis, and that in this way complements are used.

Supplement to Clinical Symptom-Complexes (Pneumonia) (p. 73).

Although there can be no doubt that during the course of pneumonia, especially at the crisis, antibodies, probably of a lytic nature, play the determining rôle, Seligmann and Klopstock† have had negative results with the complement-fixation method.

These findings are directly opposite to those of Römer ('Hand. d. Serotherapie,' 1910), and also to those of Neufeld and Händel, who discovered antibodies in the serum of pneumonia convalescents, though not in uniform amounts and not constantly.

The findings of Seligmann and Klopstock by no means

* *Zeits. f. Immun. Forsch.*, 1909, vols. ii. and iii., Nos. 6 and 7.

† *Ibid.*, 1909, vol. iv., Nos. 1 and 2.

justify a doubt as to the significance of lysins in the origin of the crisis. In the first place, according to these two authors, antibodies can be proved in Römer's pneumococcic serum by means of complement fixation. From the clinical symptoms in a pseudo-crisis we must assume that in both crises and pseudo-crises all newly-formed antibodies are utilized—*i.e.*, they are fixed to the pneumococci (the antigen). At the climax of the crisis it may even happen that a great deal of antigen is circulating in the blood. The best time for finding antibodies must necessarily be immediately preceding the crisis. Further investigations will show whether the method of complement fixation will be able to demonstrate antibodies in these cases.

Supplement to Wassermann Complement Fixation (p. 128).

Since the value of antigens varies greatly, and no antigen has been found which is entirely free from objections, it is extremely desirable to work simultaneously with several antigens of varying sensitiveness, as described below.

The variable sensitiveness of the antigen is shown as follows: There are antigens acting only mildly sensitive, often giving a negative Wassermann reaction in treated syphilis. Because these antigens are but slightly sensitive, the Wassermann reaction given by them is very certain, and positive reactions do not occur with non-specific serum. There are also antigens acting highly sensitive, with which it may sometimes happen that complement fixation—*i.e.*, Wassermann reaction—may occur even in non-specific serum.

The combining and testing of the values of antigen is, therefore, an art, and it is clear that while various investigators must have similar results in very pronounced cases, in untreated or very old cases of syphilitic infection the results may differ, especially when considered as a whole, and not recorded in the history.

It is doubtless clear from what has already been said that in standardizing the antigen a knowledge of the clinical

history of the patient from whom the serum is taken is necessary, since the interpretation of the reaction must be made according to these findings. If one is working with antigens which are controlled by an abundance of clinical material, he may, of course, arrive at a definite decision regarding sera of patients whose disease is unknown.

In setting up the Wassermann reaction the author proceeds as follows:

	Patients' Serum.	Complement.	Amboceptor + Erythrocytes.	Result.
Antigen I. 0·3	0·3	0·6		
,, II. 0·5	0·1	1·0		
,, III. 0·3	0·3	0·6 As		
,, IV. 0·5	0·1	1·0 above		
Serum —	—	0·6		
control —	0·5	0·6		

Antigens applied:

I. Alcoholic extract from human heart (Dr. Wolff-Eisner).
II. ,, specific extract.
III. ,, extract from human heart (Dr. Kirstein).
IV. Watery heart extract according to Lesser.

Positive reaction with all antigens = certain lues.
With isolated antigens positive reaction = probable lues.
All antigens negative = syphilis positively absent.

Supplement to Wassermann Complement Fixation (p. 129).

The Wassermann method has been successfully applied to confirm the presence of tapeworm, etc.

The method seems to have especial diagnostic significance in a disease where diagnosis is unusually difficult, and yet of the utmost importance from a therapeutic standpoint—*i.e.*, in the determination of echinococci. The fluid of a hydatid cyst of a sheep is used as antigen. According to all reports, the method seems certain in its results, as (1) complement fixation appears to be constant in the presence of echinococci; (2) positive complement fixation apparently does not occur in patients with other diseases; and (3), the antigen alone gives no fixation which often interferes with the Wassermann reaction and renders the result doubtful.

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